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# Germline Sequencing Identifies Rare Variants in Finnish Subjects with Familial Germ Cell Tumors

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**Purpose:** Pediatric germ cell tumors are rare, representing about 3% of childhood malignancies in children less than 15 years of age, presenting in neonates or adolescents with a greater incidence noted in older adolescents. Aberrations in primordial germ cell proliferation/differentiation can lead to a variety of neoplasms, including teratomas, embryonal carcinoma, choriocarcinoma, and yolk sac tumors.

**Patients and Methods:** Three Finnish families with varying familial germ cell tumors were identified, and whole-genome sequencing was performed using an Illumina sequencing platform. In total, 22 unique subjects across the three families were sequenced. Family 1 proband (female) was affected by malignant ovarian teratoma, Family 2 proband (female) was affected by sacrococcygeal teratoma with yolk sac tumor in the setting of Cornelia de Lange syndrome, and Family 3 proband (male) was affected by malignant testicular teratoma. Rare variants were identified using an autosomal recessive or de novo model of inheritance.

**Results:** For family 1 proband (female), an autosomal recessive or de novo model of inheritance identified variants of interest in the following genes: *CD109*, *IKBKB*, and *CTNNA3*, *SUPT6H*, *MUC5AC*, and *FRG1*. Family 2 proband (female) analysis identified gene variants of interest in the following genes: *LONRF2*, *ANO7*, *HS6ST1*, *PRB2*, and *DNM2*. Family 3 proband (male) analysis identified the following potential genes: *CRIPAK*, *KRTAP5-7*, and *CACNA1B*.

**Conclusion:** Leveraging deep pedigrees and next-generation sequencing, rare germline variants were identified that were enriched in three families from Finland with a history of familial germ cell tumors. The data presented support the importance of germline mutations when analyzing complex cancers with a low somatic mutation landscape.

**Keywords:** genomics, familial germ cell tumors, next generation sequencing, germline analysis

## Introduction

Pediatric germ cell tumors (GCT) represent about 3% of all childhood malignancies for children less than 15 years of age; this incidence increases to about 15% in adolescence and young adulthood.<sup>1</sup> These tumors may arise from the gonads or extra-gonadal tissue. There are five subtypes of pediatric GCT: teratoma, germioma, yolk sac tumor (also known as endodermal sinus tumor), embryonal carcinoma, and choriocarcinoma, as well as mixed malignant subtypes.<sup>1-3</sup> Although infantile and pediatric GCT are biologically distinct from GCT of older adolescents and adults, treatment for all GCT is essentially the same and generally grounded in large doses of platinum-based chemotherapy combined with surgery. Thus, younger patients suffer more severe side effects from treatment.<sup>2,4</sup>

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Predisposition to developing ovarian and testicular teratomas has been reported in families with multiple affected members.<sup>3</sup> While the exact mechanisms underlying GCT development remain unclear, abnormalities in the *KIT-ligand (KITLG)* and *KRAS* pathway are thought to play a role.<sup>5,6</sup> Amplifications in chromosome 12p and X chromosome have been implicated in nonseminomatous GCT. Yolk sac tumors also show gains in chromosomes 1q, 11q, 20q, as well as gains in chromosome 22; losses in chromosome 1p, 6q, and 16q have also been reported.<sup>7</sup>

There is evidence to support that methylation plays a role in GCT biology, and that methylation patterns identified in GCT can distinguish the five sub-types.<sup>8</sup> Based on prevailing literature and our experience with families enriched with germ cell malignancies, germline variant that predisposes a progenitor germ cell to malignant transformation or makes the microenvironment more permissive to tumor growth is likely to be a strong candidate for unraveling the complex biology of these cancers.

Next-generation sequencing (NGS) has significantly advanced our understanding of the genomic landscape of familial cancers.<sup>2</sup> Combining deep phenotyping with deep sequencing has the potential to identify key oncogenes involved with rare and aggressive cancers. The goal of this study was to apply these techniques to 3 independent families from Finland with an extensive history of neoplasia, but no known genomic lesions. Of interest, the people of Finland have a unique ancestry marked by a population bottleneck followed by geographic isolation on a peninsula.<sup>9</sup> Consequently, the Finnish population has an enrichment in rare genetic variants. Our study leverages the distinctive genetics of this population.

## Patients and Methods

### Subject Enrollment

Three families from Finland were enrolled for this study. The study was approved by the ethical committee of Helsinki University Hospital, Helsinki, Finland. Written informed consent was obtained from all subjects and/or their parents, as well as the relatives from whom the samples were obtained for this study. A total of 22 unique subjects were sequenced across the 3 families, n=4, n=11, and n=7, respectively (Supplemental Table 1). Family 1 proband had a malignant ovarian teratoma, diagnosed at 8 years of age, with a family history of ovarian teratoma (Supplemental Table 1). Family 2 proband was diagnosed with a neonatal sacrococcygeal teratoma and a subsequent

yolk sac tumor at 1.5 years of age, with a family history of malignancies (NOS) (Supplemental Table 1). Family 3 had a proband with a malignant testicular teratoma, diagnosed at 1 year of age, with a family history of malignancies (NOS) (Supplemental Table 1). Peripheral blood was collected from each proband and family members.

De-identified analysis of genomic data from the consented patients was conducted at Nemours (Dr. Crowgey) with IRB research determination (IRB# 1327922-1).

### Whole-Genome Sequencing (WGS) and Genome Alignment

Whole-genome sequencing was conducted at Washington University School of Medicine by the McDonnell Genome Institute. Whole-genome sequencing was conducted using the NovaSeq 6000 high-throughput sequencing platform (Illumina, San Francisco, CA) at a depth of coverage of 30X. Samples were prepared using the TruSeq whole genome library preparation (Illumina). Fastq files were processed via fastqc for data quality (Babraham Institute) and trimmed based on adaptor sequences and quality (cutadapt). Trimmed reads were mapped to the human genome hg19 via bwa mem.<sup>10</sup> Genome analysis toolkit (GATK) best practices, base quality score recalibration, indel realignment, and duplicate read removal were followed to generate a combined variant call file (VCF) using Haplotyper for all samples analyzed.<sup>11-13</sup>

### Variant Annotation and Data Analysis

VCFs were annotated using VarSeq version 2.2.0 (Golden Helix®). Specifically, variants were annotated using: ExAC Variant Frequencies 0.3, gnomAD exomes and genome variant frequencies 2.1.1,<sup>14</sup> dbSNP 151 (<http://www.ncbi.nlm.nih.gov/SNP/>), Ensemble Genes 87, and functional predictions were determined via sift,<sup>15</sup> polyphen2 HVAR,<sup>16</sup> mutation taster,<sup>17</sup> mutationAssessor,<sup>18</sup> FATHMM,<sup>19</sup> and FATHMM MKL coding. At least three of the functional predictions needed to predict the variant as damaging. Variants were filtered based on the following inheritance patterns when appropriate autosomal recessive, and de novo, using the following criteria: read depth >10, genotype qualities >20, MAF ExAC <0.01, MAF gnomAD <0.01, and effect of mutation (loss of function or missense for coding variants). The same logic was applied for non-coding variants located in introns and non-exonic (intergenic) locations.

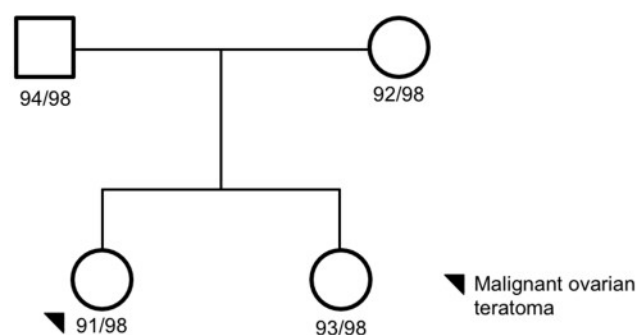
A variant was considered as autosomal recessive for proband 1 and 2 if both the mother and father were carriers

(0/1), unaffected siblings were either carriers or wildtype (0/1 or 0/0), and the proband was homozygous alternative (1/1). Additionally, for family 2 proband, the unaffected extended family members in the pedigree were used to further filter out variants if they were a homozygous alternative (1/1) genotype. Variants were considered as de novo for proband 1 and 2 if both the mother and father were homozygous wildtype (0/0), and the unaffected siblings were also homozygous wildtype (0/0). Additionally, for family 2 proband, the unaffected extended family members in the pedigree were used to further filter out variants if they were a carrier (0/1). For proband 3, unfortunately the mother's sample was not available for sequencing. The same logic as above for probands 1 and 2 was applied, except that the mother's parents' genotypes were used in replacement.

## Results

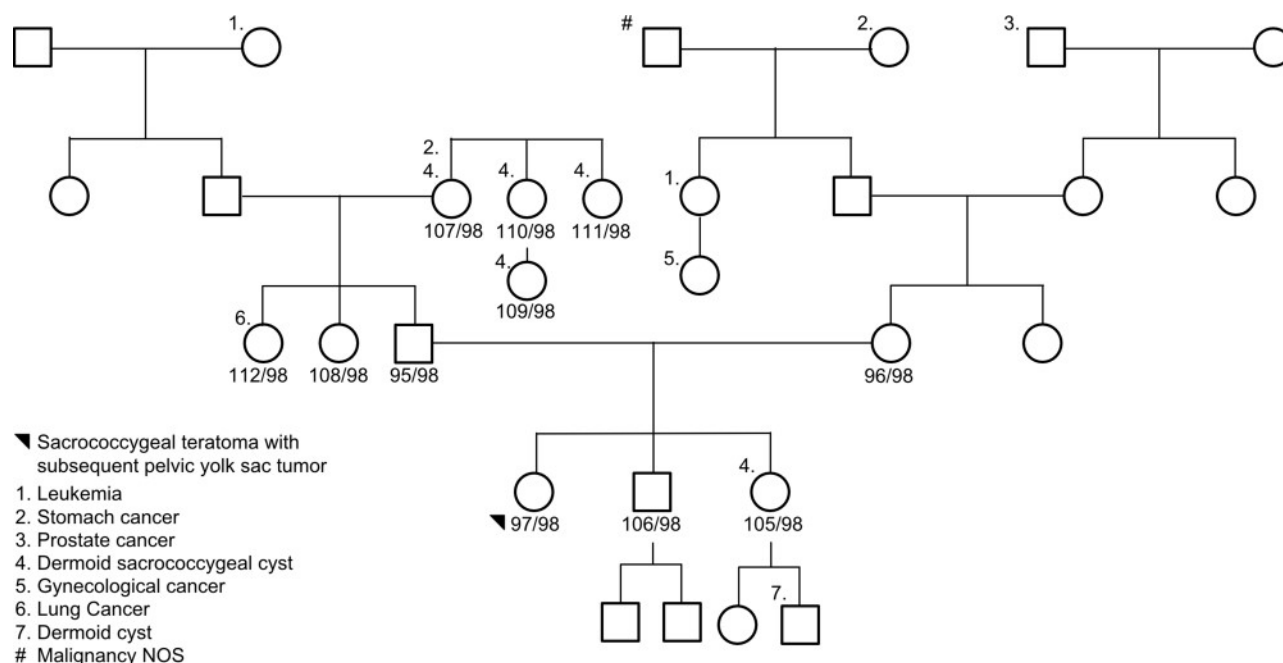
### Family 1

Four subjects were sequenced in Family 1 pedigree: mother, father, proband (ovarian teratoma), and unaffected sibling (Figure 1). A history of ovarian teratoma was noted by the primary care physician (Supplemental Table 1); however, germline samples from these extended family members were not available for sequencing. Using an autosomal recessive analysis, three variants of interest



**Figure 1** Family 1 pedigree. Four family members were sequenced via whole-genome sequencing: proband, mom, dad, and unaffected sister. The proband, black circle, was a malignant ovarian teratoma. Females are represented as circles; males are represented as squares. The number next to the pedigree represents the de-identified subject ID.

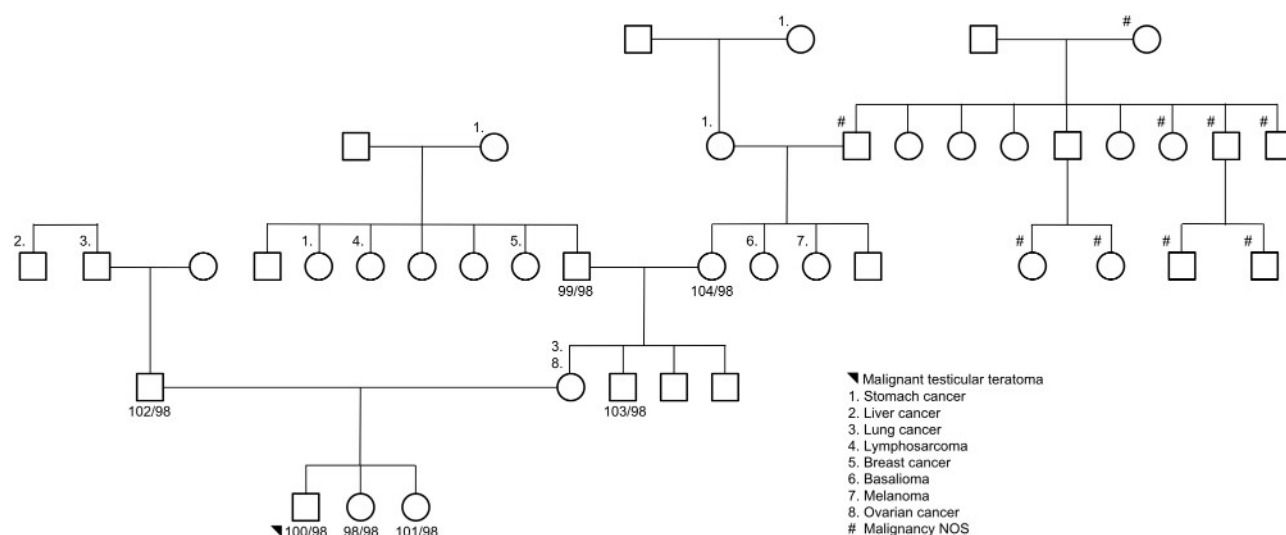
were identified in the following genes: *CD109* (rs7741152), *IKBKB* (rs140485496), and *CTNNA3* (rs192093851) (Table 1). All of the variants identified were rare within the other Finnish families analyzed with *CD109* and *IKBKB* alleles only identified in Family 1, and 1 allele count outside of Family 1 for rs192093851 (*CTNNA3*). It was noted that all three variants had a <0.01 minor allele frequency (MAF) within the gnomAD database; however, they were >0.01 within the Finnish-specific genomes within gnomAD (Table 1). Inhibitor of NF-κB kinase subunit beta (*IKBKB*) is associated with immunodeficiency disorders (IMD15B and 15A) and is a



**Figure 2** Family 2 pedigree. Eleven subjects were sequenced via whole-genome sequencing: proband, unaffected brother, unaffected sister, mother, father, two paternal aunts, paternal grandmother, 2 paternal great aunts, and a paternal cousin. The proband, black circle, was a sacrococcygeal teratoma – yolk sac tumor. Females are represented as circles; males are represented as squares. The number next to the pedigree represents the de-identified subject ID.

**Table 1** Rare Autosomal Recessive and De Novo Variants Identified in Family 1 Proband

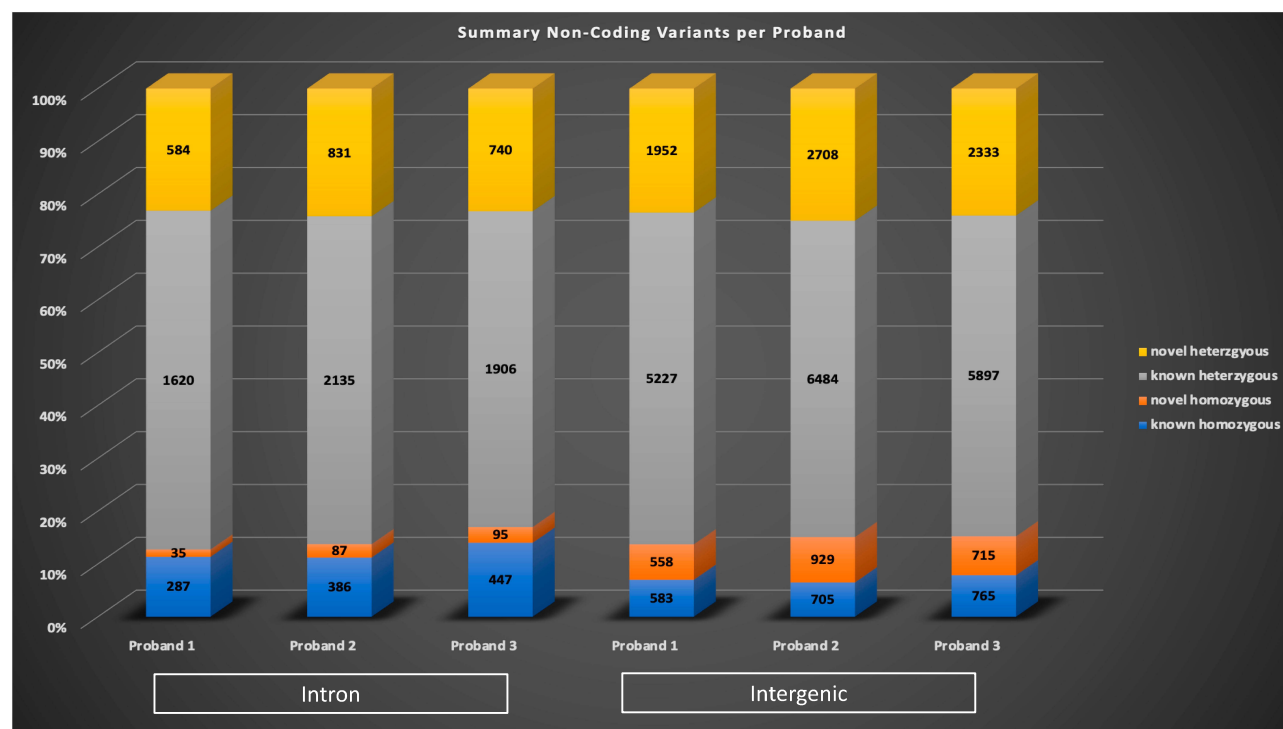
Inheritance Pattern	Chr: Position	Ref/Alt	ID	Proband 91/98	Father 94/98	Mother 92/98	Sister 93/98	Gene Names	HGVS c. (Clinically Relevant)	HGVS p. (Clinically Relevant)	Effect	GnomAD	GnomAD European Finnish
Autosomal recessive	6:747675	G/A	rs7741152	1/1	0/1	0/1	0/1	CDI09	NM_133493.4:c.1130G>A	NP_598000.2:p.Gly377 Asp	missense_variant	0.0079	0.01932
	8:42178280	C/T	rs140485496	1/1	0/1	0/1	0/1	IKBKB	NM_001556.2:c.1606C>T	NP_001547.1:p.Arg536 Trp	missense_variant	0.00373	0.02279
	10:68979466	C/A	rs192093851	1/1	0/1	0/1	0/0	CTNNA3	NM_013266.3:c.742G>T	NP_037398.2:p.Ala248 Ser	missense_variant	0.00344	0.02396
De novo	1:6505837	AG/-		0/1	0/0	0/0	0/0	ESPN	NM_031475.2:c.1306_1307de IAG	NP_113663.2:p.Ser436 Leufs	frameshift_variant	0.0011	0
	1:109268468	-/CCGTA	rs1342022852	0/1	0/0	0/0	0/0	FNDC7	NM_001144937.1:c.952_953 insCCGTA	NP_001138409.1:p.Val3 I8Alafs	frameshift_variant	0.00043	0
	1:109268470	T/A	rs1223692320	0/1	0/0	0/0	0/0	FNDC7	NM_001144937.1:c.955T>A	NP_001138409.1:p.Phe 319Ile	missense_variant	0.00041	0
	1:109268473	GTG/-	rs1248759911	0/1	0/0	0/0	0/0	FNDC7	NM_001144937.1:c.958_960 delGTG	NP_001138409.1:p.Val 320del	inframe_deletion	0.00043	0
	1:109268476	-/AAAAAA	rs1225123862	0/1	0/0	0/0	0/0	FNDC7	NM_001144937.1:c.960_961 insAAAAAA	NP_001138409.1:p. Lys321_Ser322insLys	inframe_insertion	0.00043	0
	4:190878556	-/A		0/1	0/0	0/0	0/0	FRGI	NM_004477.2:c.435_436insA	NP_004468.1:p.Met147 Asnfs	frameshift_variant	0.00158	0.003837
	11:1213565	-/A		0/1	0/0	0/0	0/0	MUC5AC	NM_001304359.1:c.2680_2681 insA	NP_001291288.1:p.Pro 894Hlfs	frameshift_variant	0.00128	0
	11:1213571	-/AACCGG	rs1300356008	0/1	0/0	0/0	0/0	MUC5AC	NM_001304359.1:c.2686_2687 insAACCGG	NP_001291288.1:p.Thr 896Lysfs	missense_variant	0.00128	0
	12:109017014	GG T/G	rs1373341486	0/1	0/0	0/0	0/0	SEPLG	NM_001206609.1:c.1118A>C	NP_001193538.1:p.Tyr 373Ser	missense_variant	0.00128	0
	17:27013754	G/A		0/1	0/0	0/0	0/0	SUPT6H	NM_003170.4:c.2647G>A	NP_003161.2:p.Glu88 3Lys	missense_variant	0.00128	0
	18:34298343	ACCTTTCT GGGTTTG/-		0/1	0/0	0/0	0/0	PHOD3	NM_001281740.2:c.308 2_309delACCTTTCTGGGTT	NP_001268669.1:p.Thr 1028_Leu1032del	inframe_deletion	0.00581	0.003076
	12:40897291	G/A	rs1276236479	0/1	0/0	0/0	0/0	MUC19	NM_173600.2:c.18907G>A	NP_775871.2:p.? 228Asn	missense_variant	0.00581	0.0009194
	14:22102317	G/T	rs200792644	0/1	0/0	0/0	0/0	OR10G2	NM_001005466.2:c.682C>A	NP_001005466.2:p.His 228Asn	missense_variant	0.00646	0
	1:151317205	G/C	rs747555214	0/1	0/0	0/0	0/0	REF5	NM_000449.3:c.352C>G	NP_000440.1:p.Arg18 Gly	missense_variant	0	0



**Figure 3** Family 3 pedigree. Seven subjects were sequenced via whole-genome sequencing: proband, unaffected siblings, data, maternal uncle, and maternal grandparents. Females are represented as circles; males are represented as squares. The number next to the pedigree represents the de-identified subject ID.

serine kinase that plays a key role in the NF- $\kappa$ B signaling pathway. Gene variants and expression data have linked *IKBKB* with prostate and ovarian cancers.<sup>20</sup> Of interest, *IKBKB* has been associated with *KRAS*, which is a known oncogene linked to GCTs.<sup>21</sup>

The de novo analysis identified 14 rare variants within proband one across 10 unique genes (Table 1). Nine of the variants had known RefSeq (rs#) identification numbers in the dbSNP database and MAF of  $>0.01$  in the gnomAD genome database as well as within the



**Figure 4** Non-coding variants identified in the probands. Rare non-coding variants were identified in introns (left bars) and non-exonic regions (intergenic, right bars) per each proband. A variant was considered to be known if it was listed in dbSNP 151. Known homozygous variants are in blue, novel homozygous variants are in orange, known heterozygous variants are in grey, and novel heterozygous variants are in yellow.



**Table 2** Rare Autosomal Recessive and De Novo Variants Identified in Family 2 Proband

Inheritance Pattern	Chr: Position	Ref/Alt	ID	Father	Mother	Proband	Sister	Brother	Aunt	Aunt	Grandma	Great aunt
				95/98	96/98	97/98	105/98	106/98	108/98	112/98	107/98	111/98
Autosomal recessive	2:100915330 2:242128114	G/T C/T	rs116702638 rs148609049	0/1 0/1	0/1 0/1	1/1' 1/1'	0/1 0/0	0/1 0/1	0/1 0/0	0/1 0/1	0/1 0/1	0/1 0/0
De novo	1:152278769 6:43155555 6:43155558	-/CC CC/- -/ACG	rs1205280854 rs1235342930 rs1217118305	0/0 0/0 0/0	0/0 0/0 0/0	0/1 0/1 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/1 0/1	0/0 0/0 0/0	0/0 0/0 0/0
	1:152278771 1:152278831	TG/- -/TTG	rs1407703398 rs1448728360	0/0 0/0	0/0 0/0	0/1 0/1	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
	2:129025758 3:195506483	C/A TGTCGGTGAC AGGAAGGGGG GTGGCGTGAC CTGTGGATGC	rs142919429 rs1560301180	0/0 0/0	0/0 0/0	0/1 0/1	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
	3:195512233	TGAGGAAC/- -/GCCGAGGAA ACGTTGGTGA CAGGAAGAC GGGTGGTGT CACCTGTGGAA	rs529636680	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0
	6:43155547 6:43155560 6:43155560 6:43155563	C/T -/GA T/C ACT/-	rs1300018649 rs1264139231 rs1489405189 rs1189505569	0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0	0/1 0/1 0/1 0/1	0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0
	11:1093483 12:11546859	GT/- -/CCTTGAGG CTGGTTGCC TCCTTGTTGG GGGTGCTCC TTGTGGCTT TCCTGGAGG AGG	rs747244421	0/0 0/0	0/0 0/0	0/1 0/1	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
	12:40876971 12:40876979 19:10908156	C/A A/C TCAGAGCTG/-	rs769109166 rs1273078187 rs1295151630	0/0 0/0 0/0	0/0 0/0 0/0	0/1 0/1 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0
	19:41811728	-/CCCCCCCCA		0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0
	19:52888076	-/ATGAGGTC AGGAGATCG AGACCATCC TGGCTAACAA GGTG		0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0

Finnish-specific genomes within gnomAD (Table 1). Of interest, *SUPT6H*, a histone chaperone, has previously been shown to control estrogen-related transcription and linked to cancer.<sup>22</sup> Additionally, *MUC5AC* has been associated with ovarian tumors<sup>23</sup> and *FRG1* expression levels have been shown to be aberrant in several cancers.

## Family 2

Eleven subjects were sequenced in Family 2 pedigree: proband (sacroccygeal teratoma – yolk sac tumor), unaffected brother with a benign sacroccygeal cyst, unaffected sister, mother, father, two paternal aunts,

paternal grandmother, 2 paternal great aunts, and a paternal cousin (Supplemental Table 1; Figure 2). Of interest, the unaffected brother and nephew (unaffected sister) have a history of benign dermoid cysts (sacroccygeal and ophthalmic area). Furthermore, the proband's great grandfather (maternal side) had prostate cancer, and a great grandmother with stomach cancer, who has a daughter with leukemia and a granddaughter with cancer. An autosomal recessive analysis identified two rare variants in the following genes *LONRF2* (rs116702638) and *ANO7* (rs148609049) (Table 2). It was noted that all three variants had a <0.01 MAF within the gnomAD database; however, they were



Great aunt	Cousin	Gene Names	HGVS c. (Clinically Relevant)	HGVS p. (Clinically Relevant)	Effect	GnomAD	GnomAD European (Finnish)
110/98	109/98						
0/1 0/1	0/0 0/0	LONRF2 ANO7	NM_198461.3:c.1444C>A NM_001001891.3:c.88C>T	NP_940863.3:p.His482Asn NP_001001891.2:p.Arg30Ter		0.008377 0.007657	0.0201 0.03571
0/0 0/0 0/0	0/0 0/0 0/0	FLG CUL9 CUL9	NM_002016.1:c.8593_8594insGG NM_015089.3:c.1686_1687delCC NM_015089.3:c.1688_1689ins ACG	NP_002007.1:p.Ala2865Glyfs NP_055904.1:p.Leu563Alafs NP_055904.1:p.Leu563_Leu5 64insArg	frameshift_variant frameshift_variant disruptive_inframe_insertion	0.003439 0.003898 0.006126	0.02396 0 0
0/0 0/0	0/0 0/0	FLG FLG	NM_002016.1:c.8590_8591delCA NM_002016.1:c.8531_8532ins CAA	NP_002007.1:p.His2864Cysfs NP_002007.1:p.Glu2844delins AspLys	frameshift_variant disruptive_inframe_insertion		
0/0 0/0	0/0 0/0	HS6ST1 MUC4	NM_004807.2:c.1214G>T NM_018406.6:c.11921_11968del GTTCTCAGCATCCACAG GTCACGCCACCCCC TTCCTGTCAACGACA	NP_004798.3:p.Ser405Ile NP_060876.5:p.Arg3974_Thr 3990delinsPro	missense_variant disruptive_inframe_deletion	0.001622	0.0008978
0/0	0/0	MUC4	NM_018406.6:c.6218_6219insTT CCACAGGTGACACCAACCG TCTTCCTGTCAACACGTTT CCTCGGC	NP_060876.5:p. Gly2076_His2077insAspThrThr ArgLeuPro ValThrAsnValSerSerAlaSerThrGly	disruptive_inframe_insertion	0.00004043	0
0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0	CUL9 CUL9 CUL9 CUL9	NM_015089.3:c.1678C>T NM_015089.3:c.1690_1691insGA NM_015089.3:c.1691T>C NM_015089.3:c.1694_1696del ACT	NP_055904.1:p.Leu560Phe NP_055904.1:p.Leu564Argfs NP_055904.1:p.Leu564Pro NP_055904.1:p.Asn565_Ser 566delinsThr	missense_variant frameshift_variant missense_variant disruptive_inframe_deletion	0.004704 0 0.004741	0 0 0.00003985
0/0 0/0	0/0 0/0	MUC2 PRB2	NM_002457.4:c.5296_5297delGT NM_006248.3:c.153_154insCCTC CTCCAGGAAAGCCACAAG GAGCACCCCCACAAGGA GGCAACCAAGCCTCAAGG	NP_002448.4:p.Val1766Thrfs NP_006239.3:p.Ser52Profs	frameshift_variant frameshift_variant		
0/0 0/0 0/0	0/0 0/0 0/0	MUC19 MUC19 DNM2	NM_173600.2:c.10217C>A NM_173600.2:c.10225A>C NM_001005360.2:c.1336-1006_13 36-998delTCAGAGCTG	NP_775871.2:p.? NP_775871.2:p.?	missense_variant missense_variant inframe_deletion	0.002807 0.001104 0.009196	0.0003193 0 0.000204
0/0	0/0	HNRNPUL1	NM_007040.5:c.2409_2410insCC CCCCCA	NP_008971.2:p.Thr804Profs	frameshift_variant		
0/0	0/0	ZNF880	NM_001145434.1:c.1242_1243ins ATGAGGTCAGGAGATCGAG ACCATCCTGGCTAACAAGGTG	NP_001138906.1:p.Lys415Metfs	frameshift_variant		

>0.01 within the Finnish-specific genomes within gnomAD (Table 1). Variant rs148609049 has previously been published as a germline variant that associates with significantly shorter survival rates in prostate cancer subjects.<sup>24</sup> Aberrant methylation patterns for LON peptidase N-terminal domain and RING finger protein 2 (*LONRF2*) have been noted in rectal adenocarcinoma. Of interest, *ANO7* is associated with aggressive prostate cancer.<sup>24</sup>

The de novo analysis identified 19 rare variants within proband two across 10 unique genes (Table 2). Sixteen of the variants were found in dbSNP with MAF of >0.01 in the gnomAD genome database and within the Finnish-specific

genomes within gnomAD (Table 2). Of interest *HS6ST1*, *PRB2*, and *DNM2* have been previously linked to cancers.<sup>25,26</sup>

### Family 3

Seven subjects were sequenced in Family 3 pedigree: proband (malignant testicular teratoma), un-affected siblings, dad, uncle (maternal), grandparents (maternal) (Supplemental Table 1; Figure 3). Two InDels were identified in this family using an autosomal recessive pattern in the following genes (Table 3): *CRIPAK* and *KRTAP5-7*. *CRIPAK* encodes for a cysteine-rich PAK1 inhibitor protein that negatively regulates *PAK1* expression. PAKs are a

**Table 3** Autosomal Recessive and De Novo Variants Identified in Family 3 Proband

Inheritance Pattern	Chr:Pos	Ref/Alt	ID	Proband	Sister	Father	Uncle	Grand mother	Sister	Grand father	Gene Names
				100/98	101/98	102/98	103/98	104/98	98/98	99/98	
Autosomal recessive	4:1388626 11:71238676	ACGTGCCGATGCGGA CTGCTGCCAGTCCAG CTGCTGTAAGCCCTG CTGCTGCCAGTCCAG CTGCTGTAAGCCCTG	rs201732211 rs1393220620	1/1' 1/1'	0/0 0/1	0/0 0/1	0/0 0/0	0/0 0/1	0/0 0/0	0/0 0/0	CRIPAK KRTAP5-7
De novo	1:152284478 1:240371009 3:195507970 3:195507971 3:195507973 4:9245723 5:140222138	A/G T/C G/A G/A A/C A/T -/CCAACTGATCTGATA TATTGTATAGTTTAATA GCTTCTCTAGTCATCT TAAACAGGGTTGG	rs199888588 rs200975594 rs201000518 rs767776470 rs200368431 rs1411951281 rs782205437	0/1 0/1 0/1 0/1 0/1 0/1 0/1	0/0 0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0 0/0	FLG FMN2 MUC4 MUC4 MUC4 USP17L17 PCDHA1
	7:97619354 8:12286291 9:140773613	A/C C/T -/ACGACACGGAGCCC TATTTTCATCGGGATCT TT	rs1390112627 rs1211097648 rs370237172	0/1 0/1 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	OCM2 FAM86B2 CACNA1B
	11:1212902 11:1213367 11:1266007 11:1266815 11:1269763 11:1605904	C/G G/A T/C G/A C/A -/CCCCCACAAGAAC CGCAGCCCCCCC	rs200292517 rs748424415 rs774768277 rs200874900 rs200531133 rs1316818204	0/1 0/1 0/1 0/1 0/1 0/1	0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0	0/0 0/0 0/0 0/0 0/0 0/0	MUC5AC MUC5AC MUC5B MUC5B MUC5B KRTAP5-1
	11:64032525 12:40882472	C/G -/AGAGACAATTGGACT ATCAGCTGGAGTGAT	rs1159825088	0/1 0/1	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	PLCB3 MUC19
	14:19378000 18:9887384 18:76754481	A/G T/C -/CCCCCCCCCCCCC CCCC	rs761048370 rs1442865736	0/1 0/1 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	OR11H12 TXNDC2 SALL3
	X:48887808	T/C	rs1286561719	0/1	0/0	0/0	0/0	0/0	0/0	0/0	TFE3

family of proteins that are involved in cytoskeletal dynamics, cell survival and proliferation and have been well associated with cancers.<sup>27</sup> The de novo analysis identified 22 rare variants in 17 unique genes. Aberrant expression levels of *CACNA1B* have previously been associated with cancers.<sup>28</sup> Of interest, *TXNDC2* is solely expressed in testis and is predicted to regulate disulfide bonds, and *TFE3* is a transcription factor linked to cancers and is ubiquitous in fetal and adult tissues.

Non-Coding Variant Analysis

Rare variants (gnomAD MAF <0.01) in non-coding regions of the genome were analyzed, introns or intergenic regions. Heterozygous variants were only considered for a proband if the parents and unaffected siblings were homozygous wildtype (0/0), and homozygous variants were only considered for a proband if the parents and unaffected siblings were homozygous wildtype or heterozygous (0/1). A variant was considered as known if it was listed in dbSNP 151. The range of intronic variants detected per

proband was 2526–3439 (left side, Figure 4), with the majority of variants per proband were known heterozygous. The range of intergenic variants detected was 8320–10826 (right side, Figure 4), with the majority of the variants also being known heterozygous.

Discussion

GCT are primarily sporadic cancers in early childhood or adolescence with a variety of subtypes. Thus, the mechanisms involved in GCT are poorly understood. The identification of families with relatively little admixture and multiple GCT cases offers an opportunity to identify novel genes that may drive or modulate tumor onset and subtype. In this study, we performed WGS on three such non-consanguineous families from Finland, where the proband in each family had a different GCT subtype. The NGS analysis revealed different genes with an autosomal recessive or de novo inheritance pattern in each family.

Family 1, proband with malignant ovarian teratoma, analysis yielded three potential autosomal recessive

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HGVS c. (Clinically Relevant)	HGVS p. (Clinically Relevant)	Effect	GnomAD	GnomAD European Finnish
NM_001012503.1:c.329_330insCTGCTGCCAGT CCAGTGTGCTAAGCCCTGCTGCTGCCAGT CCAGTGTGCTAAGCCCTG	NP_001012521.1:p. Cys132_Ser133insGlnSerSerCys CysLysProCysCysCysGlnSerSer CysCysLysProCysCysCys NP_002007.1:p.Trp962Arg NP_001292353.1:p.Leu970Pro NP_060876.5:p.Pro3494Leu NP_060876.5:p.Pro3494Ser NP_060876.5:p.Ile3493Ser NP_001243786.1:p.Lys40Met NP_061734.1:p.Ser411Thrfs	Missense Missense  missense_variant missense_variant missense_variant missense_variant missense_variant missense_variant frameshift_variant	0.001105 0.0006583  0.02648 0.002757 0.0004797 0.0004074 0.001908 0.001287 0.00042	0.002893 0  0.000609 0.0000499 0.000198 0 0.0001092 0.000861 0.0002905
NM_006188.3:c.61+2T>G NM_001137610.1:c.593G>A NM_000718.3:c.390+1_390+2insACGACACGGA GCCCTATTTTCATCGGGATCTTT	NP_001131082.1:p.Arg198Gln	splice_donor_variant missense_variant splice_donor_variant	0.00007551 0.002466 0.005113	0 0.0007554 0.003842
NM_001304359.1:c.2018C>G NM_001304359.1:c.2483G>A NM_002458.2:c.7897T>C NM_002458.2:c.8705G>A NM_002458.2:c.11653C>A NM_001005922.1:c.576_577insGGGGGGCTGCG GTTCTTGTGGGGGG NM_000932.2:c.2755C>G NM_173600.2:c.15716_15717insAGAGACAATTGG ACTATCAGCTGGAGTGAT NM_001013354.1:c.407A>G NM_001098529.1:c.908T>C NM_171999.3:c.2489_2490insCCCCCCCCCCCC CCCCG NM_006521.5:c.1589A>G	NP_001291288.1:p.Thr673Ser NP_001291288.1:p.Arg828His NP_002449.2:p.Trp2633Arg NP_002449.2:p.Arg2902His NP_002449.2:p.Arg3885Ser NP_001005922.1:p.Ser193Glyfs  NP_000923.1:p.Arg919Gly NP_775871.2:p.?  NP_001013372.1:p.Asp136Gly NP_001091999.1:p.Leu303Pro NP_741996.2:p.Ser832Profs  NP_006512.2:p.Glu530Gly	missense_variant missense_variant missense_variant missense_variant missense_variant frameshift_variant  missense_variant disruptive_inframe_ insertion missense_variant missense_variant frameshift_variant  missense_variant	0.002578 0.0006029 0.00009861 0.0006634 0.002626 0.00003881  0 0.00398 0.001197	0.00328 0.00007648 0.00004068 0.0002522 0.008074 0  0 0.0002808 0.0001793

variants of interest in the following genes: *CD109*, *IKBKB*, and *CTNNA3*. Although variants had a MAF <0.01 in the gnomAD genome database, they did have higher MAF in the Finnish-specific genome data. We hypothesize that germline variants might cause a predisposition, but these higher allele frequencies in the Finnish population indicate that a second hit/additional variant in combination would be necessary. *CD109* is highly associated with cancers<sup>29</sup> and aberrant expression patterns are associated with squamous cell carcinoma.<sup>30</sup> *IKBKB* is a potent activator of the NF- $\kappa$ B pathway, which plays a major regulatory role during developmental transitions and the ability of KRAS to transform tissues with aberrant NF- $\kappa$ B signaling is well established.<sup>31</sup> Additionally, dysregulation of microRNAs that target *IKBKB* has been associated with ovarian tumors.<sup>32</sup> The de novo variant analysis identified rare variants within the gnomAD Finnish data, with the most interesting gene candidates being *SUPT6H*, a histone chaperone, *MUC5AC* has been associated with ovarian tumors,<sup>23</sup> and *FRG1*.

An autosomal recessive analysis for family 2 yielded two potential variants of interest in the following genes: *LONRF2*, and *ANO7*. *ANO7* encodes for the protein anoc-tamin-7, which has calcium-dependent phospholipid scramblase activity. The variant detected is a loss of function variant resulting in a stop gain at amino acid 30 in the translated protein and has previously been published as a germline variant that associates with significantly shorter survival rates in prostate cancer subjects.<sup>24</sup> The de novo analysis identified rare variants within the gnomAD Finnish data and included *HS6ST1*, *PRB2*, and *DNM2*, which all have been previously linked to cancers.<sup>25,26</sup>

Family 3 had variants of interest identified in *CRIPAK* and *KRTAP5-7*. *CRIPAK* is a novel interacting partner of *PAK1*.<sup>33</sup> *PAK1*, also referred to as p21-activated kinase 1, is a complex gene involved in many cellular signaling pathways including mitosis. Recent studies have described *PAK1* has a relevant oncogene in ovarian carcinoma<sup>34</sup> and breast carcinoma,<sup>35</sup> phenotypes recorded within this family history. However, the pedigree and variant results

for Family 3's proband suggest a potential sporadic case of a neoplasm. A de novo analysis did identify rare variants in *TXNDC2*, which is solely expressed in testis and is predicted to regulate disulfide bonds, and *TFE3*, a transcription factor linked to pediatric cancers that is ubiquitously expressed in fetal and adult tissues.

A current limitation of this study is the lack of functional studies, which are required to further validate the biological significance of the variants identified. It is challenging to functionally validate these types of complex variants associated with a complex phenotype, as models are difficult to establish. Unfortunately, tumor testing, which looks for abnormalities in cancer cells, was not conducted at the time of diagnosis for the probands. These data could have provided additional insight for interpreting the germline variants identified. Furthermore, the non-coding variant analysis identified numerous variants that are difficult to interpret without additional functional testing.

## Abbreviations

GCT, germ cell tumors; NGS, next-generation sequencing; WGS, whole-genome sequencing; GATK, genome analysis toolkit; VCF, variant call file; MAF, minor allele frequency; SISu, Sequencing Initiative Suomi.

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## Author Contributions

All authors contributed to data analysis, drafting and/or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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## Disclosure

Dr Todd E Druley reports ownership, salary from ArcherDX, Inc., outside the submitted work. In addition, Dr Todd E Druley has a patent #62/106,967 pending to Canopy Biosciences. The authors report no other conflicts of interest in this work.

## References

- Olson TA, Murray MJ, Rodriguez-galindo C, et al. Pediatric and adolescent extracranial germ cell tumors: the road to collaboration. *J Clin Oncol*. 2015;33(27):3018–3028. doi:10.1200/JCO.2014.60.5337
- Pierce JL, Frazier AL, Amatruda JF. Pediatric germ cell tumors: a developmental perspective. *Adv Urol*. 2018;2018. doi:10.1155/2018/9059382.
- Variants in BAK1, SPRY4, and GAB2 are associated with pediatric germ cell tumors: a report from the Children's Oncology Group/HHS Public Access: Genes Chromosome Cancer. 2018;56(7):612–625. doi:10.1002/gcc.22457.Variants
- Acharya S, Dewees T, Shinohara ET, Perkins SM. Long-term outcomes and late effects for childhood and young adulthood intracranial germinomas. *Neuro-Oncology*. 2015;17(5):741–746. doi:10.1093/neuonc/nou311
- Ferlin A, Pengo M, Pizzol D, Carraro U, Frigo AC, Foresta C. Variants in KITLG predispose to testicular germ cell cancer independently from spermatogenic function. *Endocr Relat Cancer*. 2012;101–108. doi:10.1530/ERC-11-0340
- Olie RA, Looijenga LHJ, Boerrigter L, Top B, Rodenhuis S, Langeveld A. N- and KRAS mutations in primary testicular germ cell tumors: incidence and possible biological implications. *Genes Chromosomes Cancer*. 1995;116:110–116.
- Mostert M, Rosenberg C, Stoop H, Schuyer M, Timmer A. Comparative genomic and in situ hybridization of germ cell tumors of the infantile testis. *Lab Invest*. 2000;80(7):1055–1064.
- Rijlaarsdam MA, Tax DMJ, Gillis AJM, Dorsers LCJ. Genome wide DNA methylation profiles provide clues to the origin and pathogenesis of germ cell tumors. *PLoS One*. 2015;1–34. doi:10.1371/journal.pone.0122146
- Chheda H, Palta P, Pirinen M, et al. Whole-genome view of the consequences of a population bottleneck using 2926 genome sequences from Finland and United Kingdom. *Nat Publ Gr*. 2017;25(4):477–484. doi:10.1038/ejhg.2016.205
- Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754–1760. doi:10.1093/bioinformatics/btp324
- Auwer GA, Carneiro MO, Hartl C, et al. From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Curr Protoc Bioinformatics*. 2013;43(1). doi:10.1002/0471250953.bi1110s43
- DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet*. 2011;43(5):491–498. doi:10.1038/ng.806
- McKenna A, Hanna M, Banks E, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297–1303. doi:10.1101/gr.107524.110
- Karczewski KJ, Francioli LC, Tiao G, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. *BioRxiv*. 2019;531210.
- Sim N-L, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res*. 2012;40(W1):W452–W457. doi:10.1093/nar/gks539

16. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248–249. doi:10.1038/nmeth0410-248
17. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods*. 2014;11(4):361–362. doi:10.1038/nmeth.2890
18. Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. *Nucleic Acids Res*. 2011;39(17):e118. doi:10.1093/nar/gkr407
19. Rogers MF, Shihab HA, Mort M, Cooper DN, Gaunt TR, Campbell C. FATHMM-XF: accurate prediction of pathogenic point mutations via extended features. Hancock J, ed. *Bioinformatics*. 2018;34(3):511–513. doi:10.1093/bioinformatics/btx536
20. Page A, Navarro M. Context-dependent role of IKK  $\beta$  in cancer. *Genes*. 2017;8(12):376. doi:10.3390/genes8120376
21. Hacioglu BM, Kodaz H, Erdogan B, Cinkaya A, Tastekin E. K-RAS and N-RAS mutations in testicular germ cell tumors. *Bosn J Basic Med Sci*. 2017;17:159–163.
22. Bedi U, Scheel AH, Hennion M, Begus-Nahrmann Y, Rüschhoff J, Johnsen SA. SUPT6H controls estrogen receptor activity and cellular differentiation by multiple epigenomic mechanisms. *Oncogene*. 2015;34(4):465–473. doi:10.1038/onc.2013.558
23. Hirabayashi K, Yasuda M, Kajiwaru H, et al. Alterations in mucin expression in ovarian mucinous tumors: immunohistochemical analysis of MUC2, MUC5AC, MUC6, and CD10 expression. *Acta Histochem Cytochem*. 2008;41(2):15–21. doi:10.1267/ahc.08008
24. Kaikkonen E, Rantapero T, Zhang Q, et al. ANO7 is associated with aggressive prostate cancer. *Int J Cancer*. 2018;143(10):2479–2487. doi:10.1002/ijc.31746
25. Backen AC, Cole CL, Lau SC, et al. Heparan sulphate synthetic and editing enzymes in ovarian cancer. *Br J Cancer*. 2007;96(10):1544–1548. doi:10.1038/sj.bjc.6603747
26. Joshi HP, Subramanian IV, Schnettler EK, et al. Dynamin 2 along with microRNA-199a reciprocally regulate hypoxia-inducible factors and ovarian cancer metastasis. *Proc Natl Acad Sci U S A*. 2014;111(14):5331–5336. doi:10.1073/pnas.1317242111
27. Dummler, Bettina et al. “Pak protein kinases and their role in cancer.” *Cancer metastasis reviews*. 2009;28(1-2):51–63. doi:10.1007/s10555-008-9168-1
28. Phan NN, Wang C-Y, Chen C-F, Sun Z, Lai M-D, Lin Y-C. Voltage-gated calcium channels: novel targets for cancer therapy. *Oncol Lett*. 2017;14(2):2059–2074. doi:10.3892/ol.2017.6457
29. Hashimoto M, Ichihara M, Watanabe T, et al. Expression of CD109 in human cancer. *Oncogene*. 2004;3716–3720. doi:10.1038/sj.onc.1207418.
30. Qi R, Dong F, Liu Q, Murakumo Y, Liu J. CD109 and squamous cell carcinoma. *J Transl Med*. 2018;1–8. doi:10.1186/s12967-018-1461-3
31. Kitajima S, Thummalapalli R, Barbie DA. Seminars in cell & developmental biology inflammation as a driver and vulnerability of KRAS mediated oncogenesis. *Semin Cell Dev Biol*. 2016;58:127–135. doi:10.1016/j.semedb.2016.06.009
32. Edson MA, Nagaraja AK, Matzuk MM. The mammalian ovary from genesis to revelation. *Endocr Rev*. 2009;30:624–712. doi:10.1210/er.2009-0012
33. Talukder AH, Meng Q, Kumar R. CRIPak, a novel endogenous Pak1 inhibitor. *Oncogene*. 2006;1311–1319. doi:10.1038/sj.onc.1209172
34. Schraml P, Schwerdtfeger G, Burkhalter F, et al. Combined array comparative genomic hybridization and tissue microarray analysis suggest PAK1 at 11q13. 5-q14 as a critical oncogene target in ovarian carcinoma. *Ame J Pathol*. 2003;163(3):985–992.
35. Ong CC, Jubb AM, Haverty PM, et al. apoptosis of tumor cells. *Proc Natl Acad Sci*. 2011;118(2):2–7. doi:10.1073/pnas.1103350108

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