

# 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*, *HSST1*, *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.

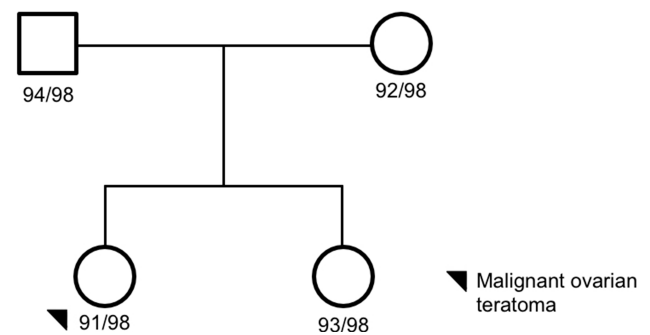
A variant was considered as autosomal recessive for proband 1 and 2 if both the mother and father were carriers

(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

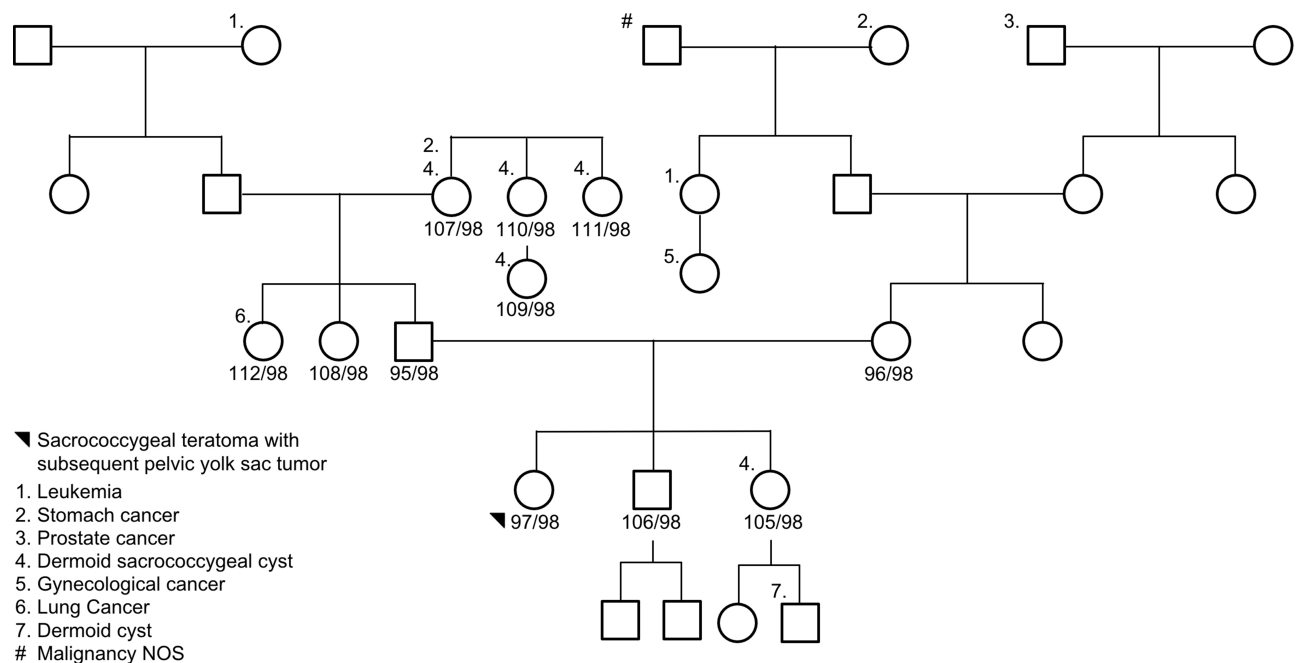
### Family 1

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, dada, 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.

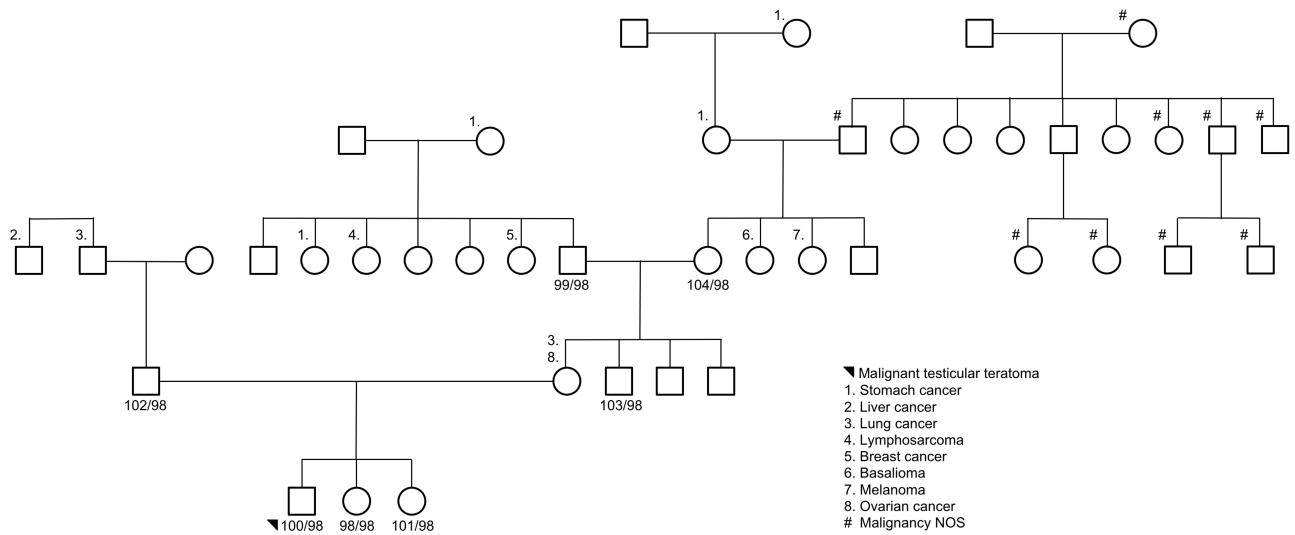
were identified in the following genes: *CD109* (rs7741152), *IKBKB* (rs140485496), and *CTNNA3* (rs192093851) (Table 1). All of the variants identified were rare within the other Finnish families analyzed with *CD109* and *IKBKB* alleles only identified in Family 1, and 1 allele count outside of Family 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

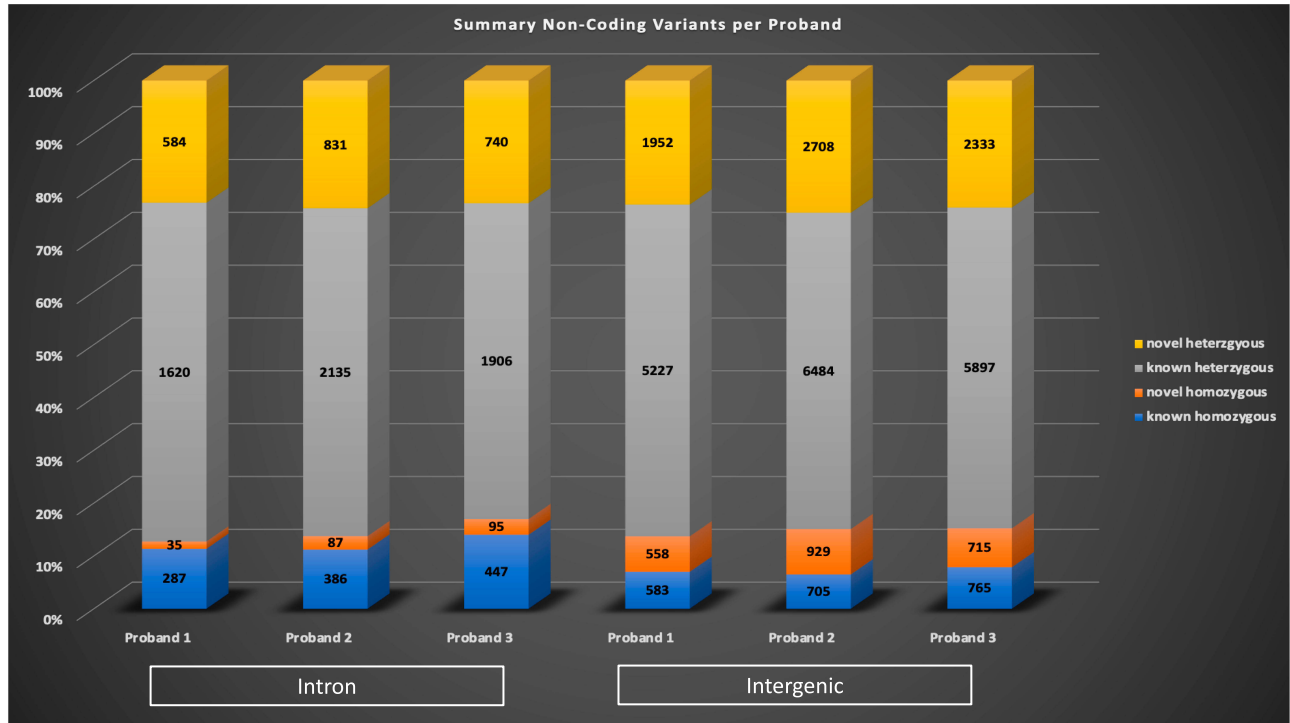
Inheritance Pattern	Chr. Position	Ref/Alt	ID	Proband 91/98	Father 94/98	Mother 92/98	Sister 93/98	Gene Names	HGVS c. (Clinically Relevant)	HGVS p. (Clinically Relevant)	Effect	GnomAD	GnomAD European Finnish
Autosomal recessive	6:7475675	G/A	rs7741152	1/1	0/1	0/1	0/1	CDI09	NM_133493.4:c.1130G>A	NP_598000.2:p.Gly377 Asp NP_001547.1:p.Arg536 Trp NP_037398.2:p.Ala248 Ser	missense_variant missense_variant missense_variant	0.0079 0.00373 0.00344	0.01932 0.02279 0.02396
	8:42178280	C/T	rs140485496	1/1	0/1	0/1	0/1	IKBKB	NM_001556.2:c.1606C>T				
	10:68979466	C/A	rs192093851	1/1	0/1	0/1	0/0	CTNNA3	NM_013266.3:c.742G>T				
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 NP_001138409.1:p.Val3 I8Alafs	frameshift_variant frameshift_variant	0.0011 0.00043	0 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.Phe 319Ile NP_001138409.1:p.Val 320del	missense_variant inframe_deletion	0.00043 0.00041	0 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. Lys321_Ser322insLysLys	missense_variant	0.00043	0
	1:109268473	GTC/-	rs1248759911	0/1	0/0	0/0	0/0	FNDC7	NM_001144937.1:c.958_960 delGTG	NP_001138409.1:p. Asnfs	inframe_insertion	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_Ser322insLysLys	frameshift_variant	0.00043	0
	4:190878556	-/A		0/1	0/0	0/0	0/0	FRG1	NM_004477.2:c.435_436insA	NP_004468.1:p.Met147	frameshift_variant		
	11:1213565	-/A		0/1	0/0	0/0	0/0	MUC5AC	NM_001304359.1:c.2680_2681 insA	NP_001291288.1:p.Pro 894Hisfs	frameshift_variant	0.00158	0.003837
	11:1213571	-/AACCCG	rs1300356008	0/1	0/0	0/0	0/0	MUC5AC	NM_001304359.1:c.2686_2687 insAACCCGGG	NP_001291288.1:p.Thr 896Lysfs	frameshift_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_309delACCTTTCTGGGTTT	NP_001268669.1:p.Thr 1028_Leu1032del	inframe_deletion		
	12:40897291 14:22102317	G/A G/T	rs1276236479 rs200792644	0/1 0/1	0/0 0/0	0/0 0/0	0/0 0/0	MUC19 ORI10G2	NM_173600.2:c.18907G>A NM_001005466.2:c.682C>A	NP_775871.2:p.? NP_001005466.2:p.His 228Asn	missense_variant missense_variant	0.00581 0.00646	0.003076 0.0009194
	1:151317205	G/C	rs747555214	0/1	0/0	0/0	0/0	RFX5	NM_000449.3:c.352C>G	NP_000440.1:p.Arg118 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-κB 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 ACGTGGTGA 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	-/CCCCCCA		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 (sacrocoxygeal teratoma – yolk sac tumor), unaffected brother with a benign sacrocoxygeal 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 (sacrocoxygeal 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 TTCTGTACCCGACA	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 CCACAGGTGACACCACCCG TCTTCTGTACCAACGTTT 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 GAGCACCCCAACAAGGA GGCAACCAGCCTCAAGG	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 ACCATCTGGCTAACAAAGGTG	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	ACGTGCCGATGCGGA	rs201732211	1/1'	0/0	0/0	0/0	0/0	0/0	0/0	CRIPAK KRTAP5-7
	11:71238676	CTGCTGCCAGTCCAG CTGCTGTAAGCCCTG CTGCTGCCAGTCCAG CTGCTGTAAGCCCTG	rs1393220620	1/1'	0/1	0/1	0/0	0/1	0/0	0/0	
De novo	1:152284478	A/G	rs199888588	0/1	0/0	0/0	0/0	0/0	0/0	0/0	FLG
	1:240371009	T/C	rs200975594	0/1	0/0	0/0	0/0	0/0	0/0	0/0	FMN2
	3:195507970	G/A	rs201000518	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC4
	3:195507971	G/A	rs767776470	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC4
	3:195507973	A/C	rs200368431	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC4
	4:9245723	A/T	rs1411951281	0/1	0/0	0/0	0/0	0/0	0/0	0/0	USP17L17
	5:140222138	-/CCAACTGATCTGATA TATTGTATAGTTAATA GCTTCTCTAGTCATCT TAAACAGGGTTGG	rs782205437	0/1	0/0	0/0	0/0	0/0	0/0	0/0	PCDHA1
	7:97619354	A/C	rs1390112627	0/1	0/0	0/0	0/0	0/0	0/0	0/0	OCM2
	8:12286291	C/T	rs1211097648	0/1	0/0	0/0	0/0	0/0	0/0	0/0	FAM86B2
	9:140773613	-/ACGACACGGAGCCC TATTTTCATCGGGATCT TT	rs370237172	0/1	0/0	0/0	0/0	0/0	0/0	0/0	CACNA1B
	11:1212902	C/G	rs200292517	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC5AC
	11:1213367	G/A	rs748424415	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC5AC
	11:1266007	T/C	rs774768277	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC5B
	11:1266815	G/A	rs200874900	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC5B
	11:1269763	C/A	rs200531133	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC5B
	11:1605904	-/CCCCCACAAGAAC CGCAGCCCCC	rs1316818204	0/1	0/0	0/0	0/0	0/0	0/0	0/0	KRTAP5-1
	11:64032525	C/G		0/1	0/0	0/0	0/0	0/0	0/0	0/0	PLCB3
	12:40882472	-/AGAGACAATTGGACT ATCAGCTGGAGTGAT	rs1159825088	0/1	0/0	0/0	0/0	0/0	0/0	0/0	MUC19
	14:19378000	A/G	rs761048370	0/1	0/0	0/0	0/0	0/0	0/0	0/0	OR11H12
	18:9887384	T/C	rs1442865736	0/1	0/0	0/0	0/0	0/0	0/0	0/0	TXNDC2
	18:76754481	-/CCCCCCCCCCCC CCCC		0/1	0/0	0/0	0/0	0/0	0/0	0/0	SALL3
	X:48887808	T/C		rs1286561719	0/1	0/0	0/0	0/0	0/0	0/0	0/0

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



HGVS c. (Clinically Relevant)	HGVS p. (Clinically Relevant)	Effect	GnomAD	GnomAD European Finnish
NM_001012503.1:c.329_330insCTGCTGCCAGT CCAGCTGCTGTAAGCCCTGCTGCTGCCAGT CCAGCTGCTGTAAGCCCTG	NP_001012521.1:p. Cys132_Ser133insGlnSerSerCys CysLysProCysCysCysGlnSerSer CysCysLysProCysCysCys	Missense Missense	0.001105 0.0006583	0.002893 0
NM_002016.1:c.2884T>C NM_001305424.1:c.2909T>C NM_018406.6:c.10481C>T NM_018406.6:c.10480C>T NM_018406.6:c.10478T>G NM_001256857.1:c.119A>T NM_018900.3:c.2394+53868_2394+53869ins CCAACTGATCTGATATATTGTATAGTTAA TAGCTTCTCTAGTCATCTTAAACAGGGTTGG	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_variant missense_variant missense_variant missense_variant missense_variant missense_variant frameshift_variant	0.02648 0.002757 0.0004797 0.0004074 0.001908 0.001287 0.00042	0.000609 0.0000499 0.000198 0 0.0001092 0.0008681 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	missense_variant missense_variant missense_variant missense_variant missense_variant frameshift_variant	0.002578 0.0006029 0.00009861 0.0006634 0.002626 0.00003881	0.00328 0.00007648 0.00004068 0.0002522 0.008074 0
	NP_000923.1:p.Arg919Gly NP_775871.2:p.?	missense_variant disruptive_inframe_ insertion	0 0	0 0
	NP_001013372.1:p.Asp136Gly NP_001091999.1:p.Leu303Pro NP_741996.2:p.Ser832Profs	missense_variant missense_variant frameshift_variant	0.00398 0.001197	0.0002808 0.0001793
	NP_006512.2:p.Glu530Gly	missense_variant		

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 anocytamin-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.

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