Germline pathogenic variants in 786 neuroblastoma patients

SUPPLEMENTARY INFORMATION

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I. SUPPLEMENTARY METHODS

Neuroblastoma patient samples

A total of 786 neuroblastoma patients accrued through the North American-based Children's Oncology Group (COG) ANBL00B1 biology study were included in the study (**Table 1**, **eTable 1**). Patients were unselected for family history of neuroblastoma. Genomic DNA was extracted from peripheral blood lymphocyte samples and obtained through the COG nucleic acids bank housed at the Children's Hospital of Philadelphia (CHOP). Matched diagnostic tumor DNA and RNA was also obtained from the same biobank. The patient cohort was intentionally enriched for high-risk disease and poor outcome through the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative. We have previously reported a small number of germline variants based on exome sequencing in a subset (n=222) of these patients[1]; however, an in-depth study of pathogenic germline variation in these children was not performed at that time.

Penn Med Biobank (PMBB)

The Penn Medicine BioBank (PMBB) is a precision medicine cohort with genomic profiling of participants who consented for biospecimen collection and linkage of their biospecimen to their electronic health record (EHR) data.[2] Starting in 2004, participants were recruited into PMBB at the time of medical appointments in the University of Pennsylvania Health System. All individuals recruited were patients of clinical practice sites of the University of Pennsylvania Health System. Appropriate consent was obtained from each participant regarding storage of biological specimens, genetic sequencing, access to all available EHR data, and permission to recontact for future studies. The study was approved by the Institutional Review Board of the University of Pennsylvania.

Phenotyping and identification of PMBB participants who were cancer-free and also had no history of a benign tumor was performed as described previously.[3] Briefly, participants with cancer were identified from the EHR using International Classification of Diseases (ICD)-9 or ICD-10 billing codes. These included both prevalent and

incident cases. We then defined a cancer- and tumor-free control cohort as individuals with no ICD-9/10 codes for invasive cancer, benign, in situ, or secondary neoplasms (n=6,295).

In this study, we included 6,295 individuals who had undergone whole-exome sequencing (WES) and germline genome-wide DNA array-based genotyping. For each individual, DNA was extracted from stored buffy coats and then exome sequences were generated by the Regeneron Genetics Center (Tarrytown, NY) and obtained for study. For purposes of this study, all WES data were reprocessed as described above for neuroblastoma data. Germline genome-wide DNA array-based genotyping using the Infinium Global Screening Array (GSA) chip (Illumina) was utilized to infer ancestry of the 6,295 individuals in the cancer- and tumor-free control cohort. Genotyping was performed at the Children's Hospital of Philadelphia or Regeneron Genetics Center as previously described.[3]

Ancestry inference

Paired germline single nucleotide polymorphism (SNP) array data were utilized to infer ancestry of neuroblastoma and Penn Medicine Biobank (PMBB) control subjects. Genotypes from neuroblastoma cases were intersected with data form the International HapMap Project (HapMap v3, draft release 2). The variants were pruned using a window size of 50 variants, step size of five variants, and pairwise r^2 threshold of 0.2. A principal component analysis (PCA) was performed using PLINK 1.9 and ancestry inferred as previously described[4]. SNP array data from PMBB samples were processed in an identical manner. Neuroblastoma SNP array data are available through the Database of Genotypes and Phenotypes (dbGaP), accession phs000124.v3.p1.

Genomic sequencing

Genomic DNA was sequenced using a combination of Complete Genomics whole genome sequencing (WGS), Illumina whole exome sequencing (WES), and Illumina targeted capture sequencing (CAP). Complete Genomics WGS (n=134) and Illumina WES (n=222) of matched tumor-normal DNA pairs was generated through the TARGET initiative (https://ocg.cancer.gov/programs/target/data-matrix), as previously described[1,5]. A total of 76

tumor-normal pairs were profiled by both WES and WGS, allowing for internal validation (**eTable 1**). An independent set of tumor-normal pairs (n=499) were sequenced by Illumina-based targeted capture sequencing within the TARGET project. Since this TARGET capture panel was designed to include genes known to be somatically mutated in childhood cancer, we constructed a new custom capture panel to include known cancer predisposition genes, neuroblastoma syndrome-related genes, and candidate susceptibility genes identified by GWAS (n=166 genes; **eTable 2**). We performed CAP germline-only sequencing of 489 of the 499 neuroblastoma cases with DNA available. For this CAP sequencing, 200 ng genomic DNA from peripheral blood lymphocyte samples was purified using Agencourt AMPure XP Reagent (Beckman Coulter Inc, Brea, CA, USA) and a next-generation sequencing library was prepared with the KAPA HyperPlus Kit (KAPA Biosystems, Wilmington, MA) using Bioo Scientific NEXTflex™ DNA Barcoded Adapters (Bioo Scientific, Austin, TX, USA) according to KAPA-provided protocol. Libraries were barcoded, amplified, pooled, and captured using NimbleGen's SeqCap EZ Choice Library with custom-designed 523-gene panel (Roche NimbleGen, Inc., Madison, WI, USA). Captured pooled libraries were amplified, cleaned, quantified, and sequenced on a HiSeq2000 using 2x125 bp paired-end sequencing protocols (Illumina, San Diego, CA, USA). Average depth of coverage for targeted capture regions across all subjects was 198x. Concordance with TARGET CAP germline sequencing was assessed for genes that overlapped in the custom captures.

Germline variant calling and annotation

Variants for Complete Genomics WGS data were produced from Complete Genomics pipeline (v2) in hg19 and single nucleotide variants (SNVs) were filtered as previously described[6]. A decision tree classifier was designed and implemented to retain high-confidence small insertion and deletion (indel) calls from this pipeline. Illumina WES (neuroblastoma and PMBB controls) and CAP (neuroblastoma) data were aligned to hg19 using Burrows Wheeler Aligner (BWA)[7] v0.7.17. Duplicates were removed using Picard v2.18.17 (http://broadinstitute.github.io/picard/) and base quality was adjusted with GATK v 4.1.6. SNVs and indels were called using HaplotypeCaller in GATK and variant quality control filtering was applied as per Broad Institute standard best practices. Resulting variants were annotated using SnpEff[8] (v4.3t) and ANNOVAR[9] (2019Oct24).

Germline variants in 166 cancer predisposition genes present on all sequencing platforms were further analyzed. Variants with read-depth coverage ≥ 15, variant allele fraction ≥ 0.20, and observed in < 0.1% across each population in the public control databases non-TCGA ExAC (exonic) or gnomAD v2.1 (non-exonic, splicing) were included in the study.

Assessment of variant pathogenicity

Pathogenicity of retained variants was assessed *in silico* using custom software to evaluate ClinVar (11-25-2020) and a modified execution of InterVar (11-25-2020) (eFigure 1). First, ClinVar calls were considered in a hierarchical manner: (1) expert panel decision, (2) consensus of "badged labs", when available. Badged labs were defined as clinical laboratories meeting minimum requirements for data sharing to support quality assurance, as defined by ClinGen (https://www.clinicalgenome.org/tools/clinical-lab-data-sharing-list/ downloaded 12-2020). Next, prior to running InterVar, we adjusted PP5 based on this modified ClinVar assessment and corrected PVS1 using AutoPVS1[10]. Variants were then assigned to be pathogenic (P), likely pathogenic (LP), benign (B), likely benign (LB) or variants of unknown significance (VUS) by first considering the adjusted ClinVar results and then the modified InterVar output. This approach was applied to both neuroblastoma cases and controls without cancer samples (PMBB and gnomAD 2.1).

Sanger sequencing

To verify germline variants, primers were designed with Primer3 and PCR reactions were carried out on 25 ng of DNA using optimized conditions for each reaction. Products were checked via gel electrophoresis. If the product had multiple bands, the entire remaining sample was run out then bands of interest excised and the DNA extracted using MinElute Gel Extraction Kit from Qiagen. Products with single bands were cleaned up and prepared for sequencing using the MinElute PCR Purification Kit (Qiagen). Samples were sequenced with two picomoles of the same primer used to create the amplicon.

Germline copy number variant (CNV) analyses

Rare germline CNVs affecting *BARD1* were identified using a SNP genotyping dataset of 5,585 neuroblastoma patients and 23,505 cancer-free control children as previously described[11]. Briefly, patients diagnosed with neuroblastoma or ganglioneuroblastoma were recruited through the Children's Oncology Group (COG) ANBL00B1 biology study without selection for clinical presentation. Germline DNA was isolated from peripheral blood or bone marrow mononuclear cells at time of diagnosis. Control children were recruited through the Children's Hospital of Philadelphia (CHOP) and screened for cancer and severe neurological or immunological disorders. Cases and controls were genotyped at the CHOP Center for Applied Genomics on matched Illumina SNP arrays and filtered for cryptic relatedness. CNVs were called using Nexus Copy Number 8.0 with linear correction for GC content, requiring a minimum of 10 probes per CNV. B-Allele Frequency (BAF) and Log R Ratio (LRR) plots were visually inspected to rule out potential artifacts.

Somatic variant analyses

Subjects harboring a germline P-LP variant in a cancer-predisposition gene were further assessed using matched tumor sequencing data when available. Specifically, somatic variants previously reported by Brady and colleagues[12] and additional variants from CAP sequencing data generated through TARGET were interrogated to assess potential second hits involving somatic SNVs, translocations, and focal copy number variations. For the TARGET CAP data, 499 tumor-normal pairs were analyzed. Somatic SNV calling was performed using Mutect 1.1.4 and annotated using Variant Effect Predictor (VEP). Somatic copy number variant (CNV) calling was performed using an in-house tool called VisCap, (https://github.com/pughlab/VisCapCancer). Tumor and normal bam files were first run through depth of coverage tool by GATK v3.0.0 and depth of coverage output was used as input for the VisCap somatic copy number calling. The calls for CNV were categorized into focal, subgenic and broad. A focal copy number variation is a case where the whole gene has gone through a copy number change. A subgenic change is when a few exons of the genes has copy number change and a broad change is for multiple genes undergoing copy number change. As not all of CPGs were included in somatic CAP sequencing for TARGET, we evaluated tumor DNA sequencing from a total of 79 subjects with germline P-LP

variant in CPGs (20 cases with capture sequencing and all (n=59) cases with WES and/or WGS tumor data) were assessed for single nucleotide variation. However, all cases with germline P-LP variants in CPGs were included in tumor DNA copy number analyses.

Statistical analyses

The prevalence of P-LP germline variants in cancer predisposition genes was reported for the neuroblastoma cohort as a whole and within clinical and biological subsets. Fisher's exact test was used to compare clinical characteristics of patients with and without P-LP germline variants in the genes studied. A two-sided Fisher's exact test (p-value < 0.05) was considered significant. Enrichment testing of P-LP variants in cases vs. controls was performed at the overall, gene, and pathway level using Fisher's exact and corresponding odds ratios (ORs) and 95% confidence intervals were computed. Where zeros cause problems with computation of the odds ratio or its confidence intervals, the Woolf logit approach was utilized. A Bonferroni correction was applied to account for multiple testing at the gene level. Kaplan-Meier analyses of event-free and overall survival were performed to compare outcomes of patients with and without germline P-LP variants. A log-rank p-value < 0.05 was considered to be statistically significant. Statistical analyses were performed using R, version 3.3.2 with R Studio, version 1.0.136. Multivariate analyses were performed with a Cox proportional-hazards regression model to identify variables that were independently predictive of outcome. A p-value < 0.05 was considered to indicate statistical significance. Variables considered included: presence of germline P-LP variant, age at diagnosis, INSS stage, *MYCN* amplification status, and risk group. The patient cohort analyzed (n=774) included all those for whom complete data were available for the variables in the model.

II. SUPPLEMENTARY TABLES

eTable 1. Neuroblastoma patient clinical, tumor biologic and sequencing data (Excel file)

This gene panel was designed to capture known cancer predisposition genes (CPGs), genes implicated in neuroblastoma-associated syndromes (syndrome), genes identified through the neuroblastoma genome-wide association study (WGS). CPG: Cancer Predisposition Gene. Chromosome location: hg19.

eTable 2. Cancer predisposition genes panel (n=166) used in study (Excel File)

This gene panel was designed to capture known cancer predisposition genes (CPGs), genes implicated in neuroblastoma-associated syndromes (syndrome), genes identified through the neuroblastoma genome-wide association study (WGS). CPG: Cancer Predisposition Gene. Chromosome location: hg19.

eTable 3. Cancer predisposition gene P-LP variants observed in neuroblastoma (Excel File)

eTable 4. Neuroblastoma cases harboring multiple germline P-LP variants in CPGs

Subject Identifier	COG Risk Group	*MYCN* status	Sex	Gene	Variant	Pathogenicity
PAPRXW	High	Amplified	Male	*ERCC2* *FANCA* *GJB2*	NM_000400:c.C2150G:p.A717G NM_001286167:c.189+1G>T NM_004004:c.G416A:p.S139N	P: ClinVar P: InterVar P: ClinVar
PARSEA	High	Not Amplified	Male	*BARD1* *ERCC5*	NM_000465:c.C448T:p.R150X NM_000123:c.2604_2605del:p.T868fs	P: ClinVar LP: InterVar
PASNEF	High	Not Amplified	Female	*BRCA1* *BRCA2*	NM_007297:c.G5101T:p.G1701C NM_000059:c.G3922T:p.E1308X	LP: ClinVar P: ClinVar
PATPXJ	High	Not Amplified	Female	*DOCK8* *GBA*	NM_001190458:c.3654delC:p.D1218fs NM_001005741: c.-50G>C	LP: InterVar P: InterVar
PATVTL	High	Amplified	Male	*BRCA2* *MSH3*	NM_000059:c.5067dupA:p.A1689fs NM_002439:c.2759delC:p.S920fs	P: ClinVar P: ClinVar
PAUICI	High	Not Amplified	Female	*IKZF1* *PHOX2B*	NM_001291845:c.T479A:p.L160X NM_003924:exon3:c.430-2A>G	LP: InterVar P: InterVar

eTable 5. Primers for Sanger sequencing validation germline variants

Gene	Subject	Location (hg19)	Type	Ref	Alt	Forward Primer	Reverse Primer
ATM	PARNCW	11:108165753	missense	G	T	CATTGTAGGGTTTGCAGTGGA	TGGCAGAGGATGAATAAAACAGG
BARD1	PATGWT	2:215595215	stop gain	C	T	TCCTGGCTTAGGTTTTTCAGA	GCAATGTTCAAGATGCCAAA
BARD1	PASFDU	2:215610566	stop gain	G	T	GATGCCCTGGGTATAGAGAGC	TCTACCCCACCTCCCAAAATTC
BARD1	PATHJZ	2:215645738	frameshift	T	---	GAGGGCTAAAAAGGCTTCTGC	TTTCTGAGGGCACCGTTTGC
BARD1	PARSEA	2:215646150	stop gain	C	T	AAATTCTTCGGGAGCTCCATGTG	TCAGAAACATCTGCAGGAGGAC
BARD1	PAHYWC	2:215657051	stop gain	C	T	AAGTGACTGCATTGGAACTGG	ATTCCAGAACTCCAGATAGATGTTT
BRCA2	PAPZYP	13:32937375	missense	A	G	ACACTGCTGTTCTCCTGTCA	ACAGCATACCACCCATCTGT
CHEK2	PARNEE	22:29121242	missense	G	A	CGCCCAGCAACTTACTCATC	GCCCTCTGATGCATGCTTTT
ERCC2	PAPZYP	19:45868346	missense	G	A	GGGCATCAAATTCCTGGGAC	AAGTTGTCCAAAACCCCAGC
EZH2	PASEGA	7:148511124	deletion	CAAGT	C	GGGTGCATTACCCAGAGAAA	AGGTGGTTGTGAGGGTTGAG
EZH2	PASTXV	7:148515025	deletion	C	---	AAATCCAATCGGCAAAACAC	AGAACTTTGCCCTGATGTTGA
EZH2	PAVCJZ	7:148544391	deletion	ATG	---	ATTTAGGGAGGCATTTCTGC	TGGCCGCAATTTAGTGTAGA
EZH2 (Somatic)	PASEGA	7:148525988-7:148531881	deletion	5893bp	---	CCACCCTACCTGGCCATAAT	ATTAAGCTCACGGGTGTTGC
FANCD2	PASJYB	3:10076378	splice	G	C	ACACCCTTCCTATCCCAAAGT	TGAAACAACTGTGCTCTCCC
WRN	PARACS	8:30924657	missense	C	G	GAAGGCTATCTGTGGGTTGTATT	AGCCTGGATTTATTAGCCTTTCA

eTable 6. Gene-based testing for excess burden of P-LP variants vs. PMBB (Excel File)
eTable 7. Gene-based testing for excess burden of P-LP variants vs. gnomAD 2.1 (Excel File)

eTable 8. P-LP variants in genes with excess burden in neuroblastoma.

Gene[a]	Subject Identifier	Gender	COG Risk Group	*MYCN* status	Variant[b]	AA Change	Variant Type
ALK	PANYGR	Male	High	Not Amplified	c.3749T>C	I1250T	Missense
ALK	PARVLK	Female	High	Not Amplified	c.3824G>A	R1275Q	Missense
ALK	PATDVF	Female	Intermediate	Not Amplified	c.3824G>A	R1275Q	Missense
BARD1	PARSEA	Male	High	Not Amplified	c.448C>T	R150X	Stopgain
BARD1	PATGWT	Male	High	Amplified	c.1921C>T	R641X	Stopgain
BARD1	PAHYWC	Male	High	Amplified	c.334C>T	R112X	Stopgain
BARD1	PATHJZ	Female	Intermediate	Not Amplified	c.860_861del	E287fs	Frameshift
BARD1	PASFDU	Female	High	Not Amplified	c.1690C>T	Q564X	Stopgain
BARD1	PASGEE	Male	High	Not Amplified	c.1677+1G>T	-	Splice
BARD1	PATZRU	Male	High	Not Amplified	c.159-1G>T	-	Splice
BARD1	PASCIX	Male	High	Not Amplified	c.1954_1955insTGAACAGGAAGAAAAGTATG	E652fs	Frameshift
EZH2	PASEGA	Male	High	Not Amplified	c.1774_1777delACTT	T592fs	Frameshift
EZH2	PASTXV	Male	Low	Not Amplified	c.1184delG	G395fs	Frameshift
EZH2	PAPVXS	Male	High	Not Amplified	c.625G>A	D209N	Missense
EZH2	PAVCJZ	Male	High	Not Amplified	c.-1_2delCAT	M1del	Start Lost

eTable 9. Rare germline copy number variants (CNVs) disrupting *BARD1* in neuroblastoma cases

SNP array CNV calls

Sample	CN Change	Estimated Size	Estimated Region	Min Length	Min Region (hg19)	Max Length	Max Region	Probe Median	Probe Count
PAVNLD	CN Loss	168822	chr2:215487324-215656145	161451	chr2:215489130-215650580	176192	chr2:215485519-215661710	-0.39509578	36
PAWNMH	CN Loss	182685	chr2:215549453-215732137	153593	chr2:215562577-215716169	211777	chr2:215536329-215748105	-0.436415702	39
PALXTB	CN Loss	70165	chr2:215623659-215693823	49912	chr2:215627397-215677308	90418	chr2:215619921-215710338	-0.707099229	16

Validation: WGS (Complete Genomics) Structural Variant Calls

Sample	SV Type	Size	Region (hg19)	Strand	Mate Pair Count	Frequency In Baseline Genome Set
PALXTB	Deletion	61875	chr2:215622570-215684445	+	20	0

Validation: WGS (Complete Genomics) Copy Number Variation Calls

Sample	CNV Type	Size	Region (hg19)	Average Coverage	Relative Coverage	CNV Score (Phred-Scaled)
PALXTB	CN Loss	62000	chr2:215622000-215684000	25	0.5	53

eTable 10. Association of P-LP variants with clinical and tumor characteristics (Full Cohort)

Characteristic	Without P-LP Variants (n=677) # subjects	% subjects	With P-LP variants (n=109) # subjects	% subjects	P-value
Sex					
Male	384	57%	63	58%	0.9171
Female	293	43%	46	42%	
Age at Diagnosis					
< 18 months	206	30%	36	33%	0.5780
> 18 months	471	70%	73	67%	
COG Risk Group					
Low	91	13%	12	11%	
Intermediate	100	15%	19	17%	0.6494
High	486	72%	78	72%	
INRG Stage					
Stage L1	77	16%	8	12%	
Stage L2	72	15%	10	15%	0.6157
Stage MS	39	8%	3	5%	
Stage M	295	61%	44	68%	
INSS Stage					
Stage 1	34	5%	4	4%	
Stage 2a	21	3%	3	3%	
Stage 2b	35	5%	1	1%	0.2187
Stage3	83	12%	10	9%	
Stage 4	460	68%	86	79%	
Stage 4s	44	6%	5	5%	
MYCN status					
Amplified	201	30%	23	21%	0.0672
Not amplified	467	70%	85	79%	
Histologic Classification					
Favorable	180	27%	31	28%	0.5491
Unfavorable	455	67%	67	61%	
unknown	42	6%	11	10%	
Degree of Differentiation					
Differentiated	30	5%	5	5%	
Undifferentiated or poorly differentiated	572	95%	89	95%	0.8023
Mitosis Karyorrhexis Index					
Low	228	39%	35	39%	
Intermediate	167	29%	33	37%	0.2276
High	184	32%	22	24%	
Ploidy					
Diploid	251	38%	42	39%	0.8307
Hyperdiploid	408	62%	65	61%	
1p LOH					
Yes	193	34%	27	33%	>0.9999
No	377	66%	54	67%	
11q LOH					
Yes	129	23%	29	36%	0.0119*
No	438	77%	51	64%	

eTable 11. Association of P-LP variants with clinical and tumor characteristics (Capture Only)

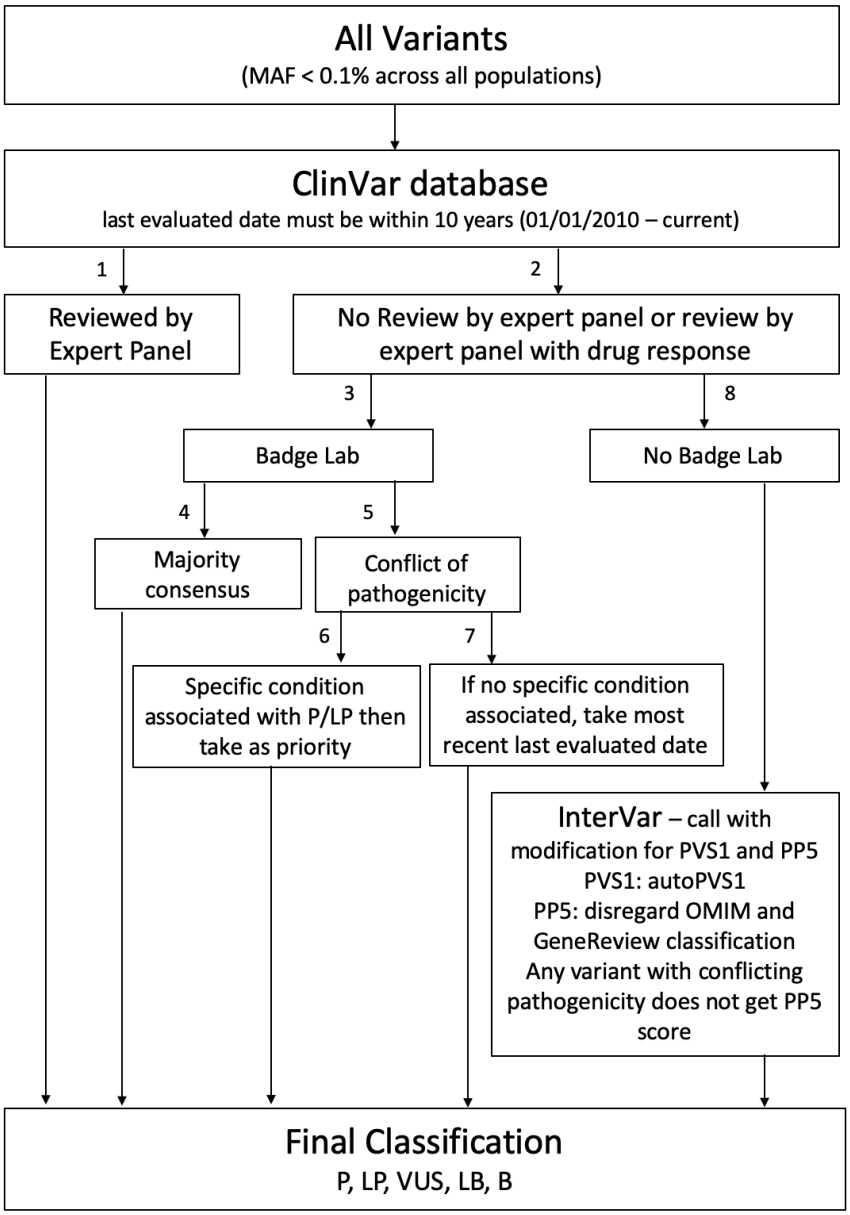
Characteristic	Without P-LP variants (n=434) # subjects	% subjects	With P-LP variants (n=55) # subjects	% subjects	P-value
Sex					
Male	234	54%	33	60%	0.4727
Female	200	46%	22	40%	
Age at Diagnosis					
< 18 months	180	41%	30	55%	0.0822
> 18months	254	59%	25	45%	
COG Risk Group					
Low	80	18%	9	16%	
Intermediate	87	20%	18	33%	0.0962
High	267	62%	28	51%	
INRG Stage					
Stage L1	76	20%	8	16%	
Stage L2	70	18%	10	20%	0.4961
Stage MS	26	7%	1	2%	
Stage M	212	55%	31	62%	
INSS Stage					
Stage 1	34	8%	4	7%	
Stage 2a	21	5%	3	5%	
Stage 2b	34	8%	1	2%	0.4352
Stage 3	76	18%	10	18%	
Stage 4	243	56%	36	65%	
Stage 4s	26	6%	1	2%	
MYCN status					
Amplified	128	30%	9	17%	0.0536
Not Amplified	303	70%	45	83%	
Histologic Classification					
Favorable	154	35%	25	45%	0.0954
Unfavorable	264	60%	26	47%	
unknown	16	4%	4	7%	
Degree of Differentiation					
Differentiated	19	5%	3	6%	0.7283
Undifferentiated or poorly differentiated	394	95%	50	94%	
Mitosis Karyorrhexis Index					
Low	164	41%	21	41%	
Intermediate	111	28%	20	39%	0.1239
High	126	31%	10	20%	
Ploidy					
Diploid	151	36%	18	33%	0.7645
Hyperdiploid	270	64%	36	67%	
1p LOH					
Yes	142	33%	19	35%	0.7624
No	284	67%	35	65%	
11q LOH					
Yes	79	19%	17	31%	0.0309*
No	346	81%	37	69%	

eTable 12. Cox proportional hazards regression model results.

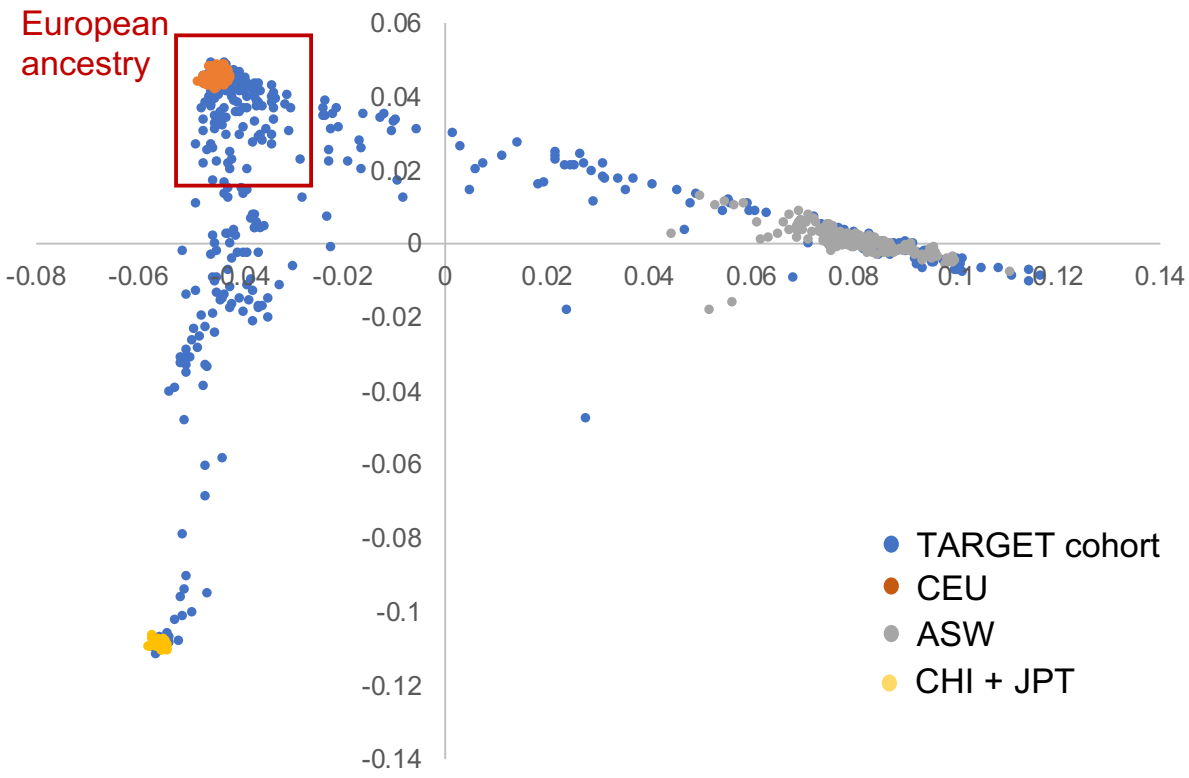
Variable[1]	Coefficient (95% Confidence Interval)	P-value	Hazard Ratio (95% Confidence Interval)
P-LP Variant	0.3677 (0.0648-0.6706)	0.0174	1.4444 (1.0699-1.9554)
Age at Diagnosis	0.0000 (-0.0001-1.0001)	0.9251	1.0000 (0.9999-1.0001)
Stage 4	1.3102 (0.7826-1.8378)	< 0.0001	3.7069 (2.1871-6.2825)
MYCN amplification	0.2610 (0.0021-0.5199)	0.0481	1.2982 (1.0021-1.6818)
Risk Group	1.0101 (0.5363-1.4839)	< 0.0001	2.7459 (1.7097-4.4100)

[1] Model based on neuroblastoma patients with data on all variables (n=774).

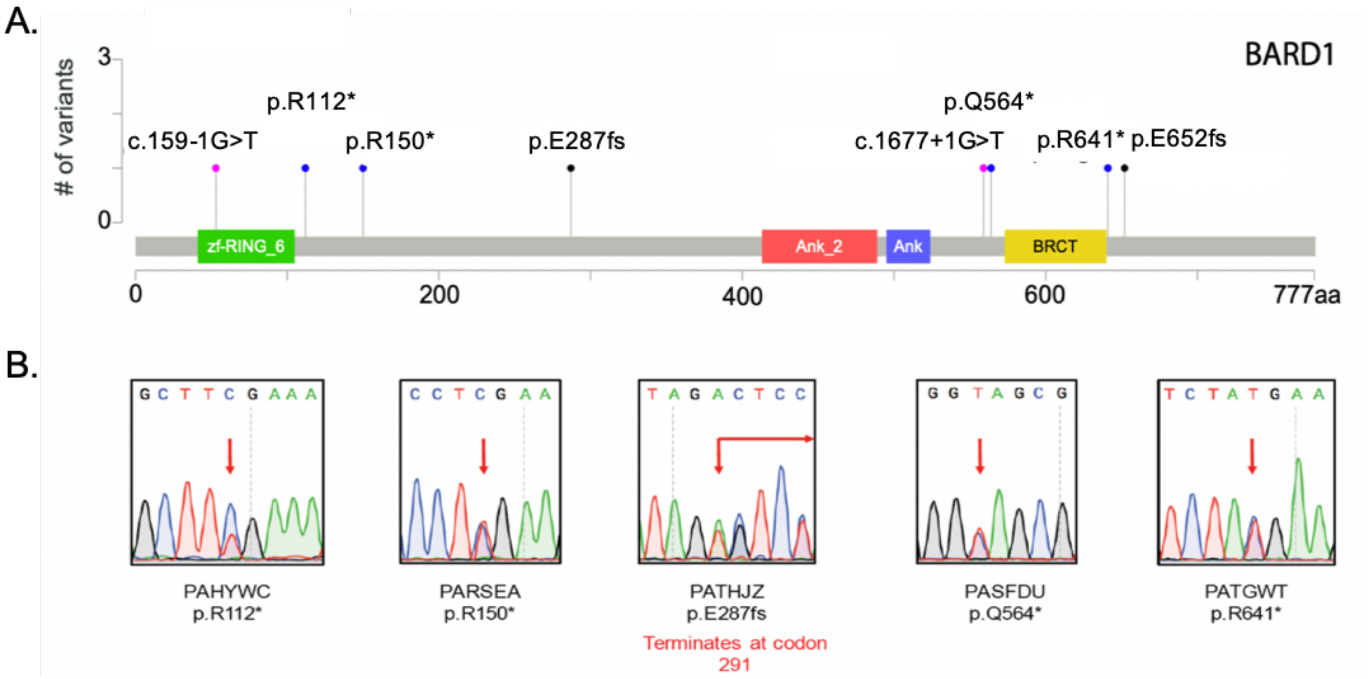
III. SUPPLEMENTARY FIGURES



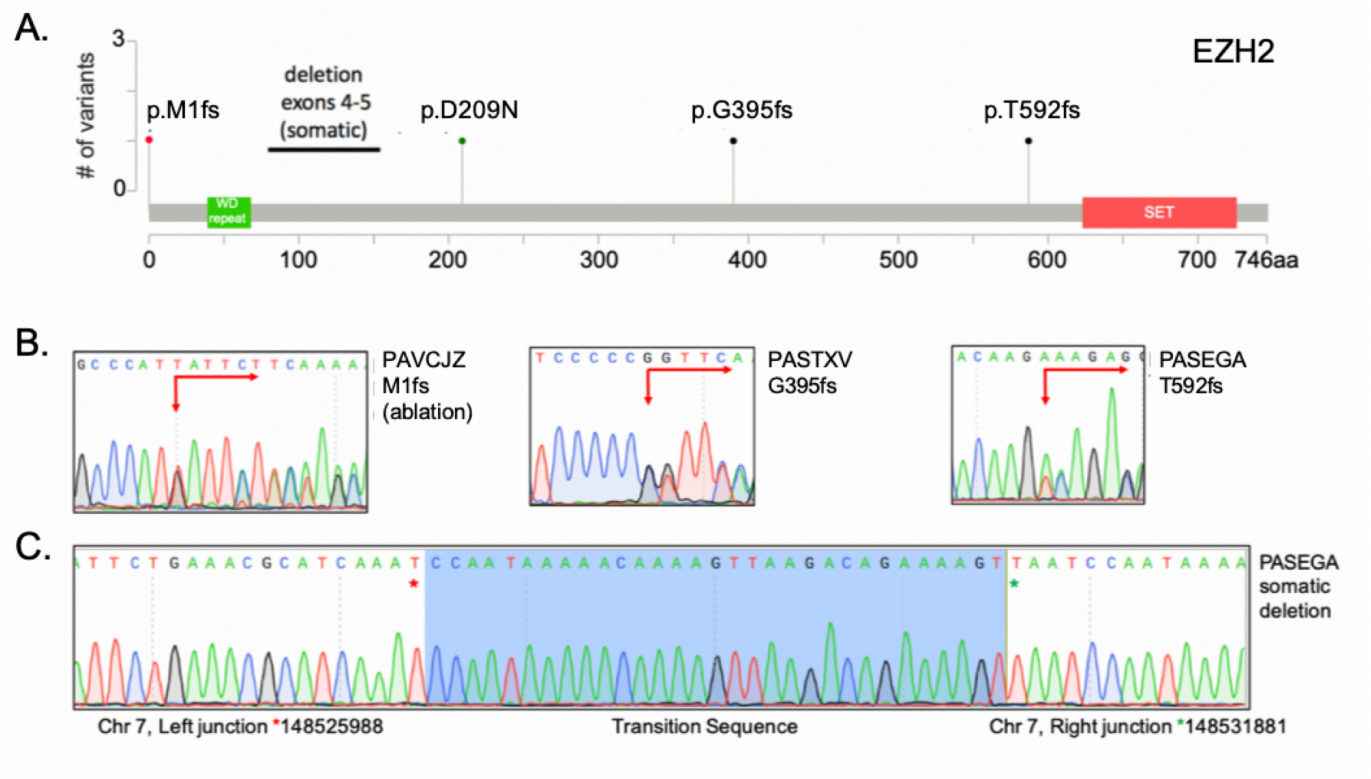
eFigure 1. **Flow diagram of automated pathogenicity assessment for germline variants.**
Order of flow indicated by labeled arrows.



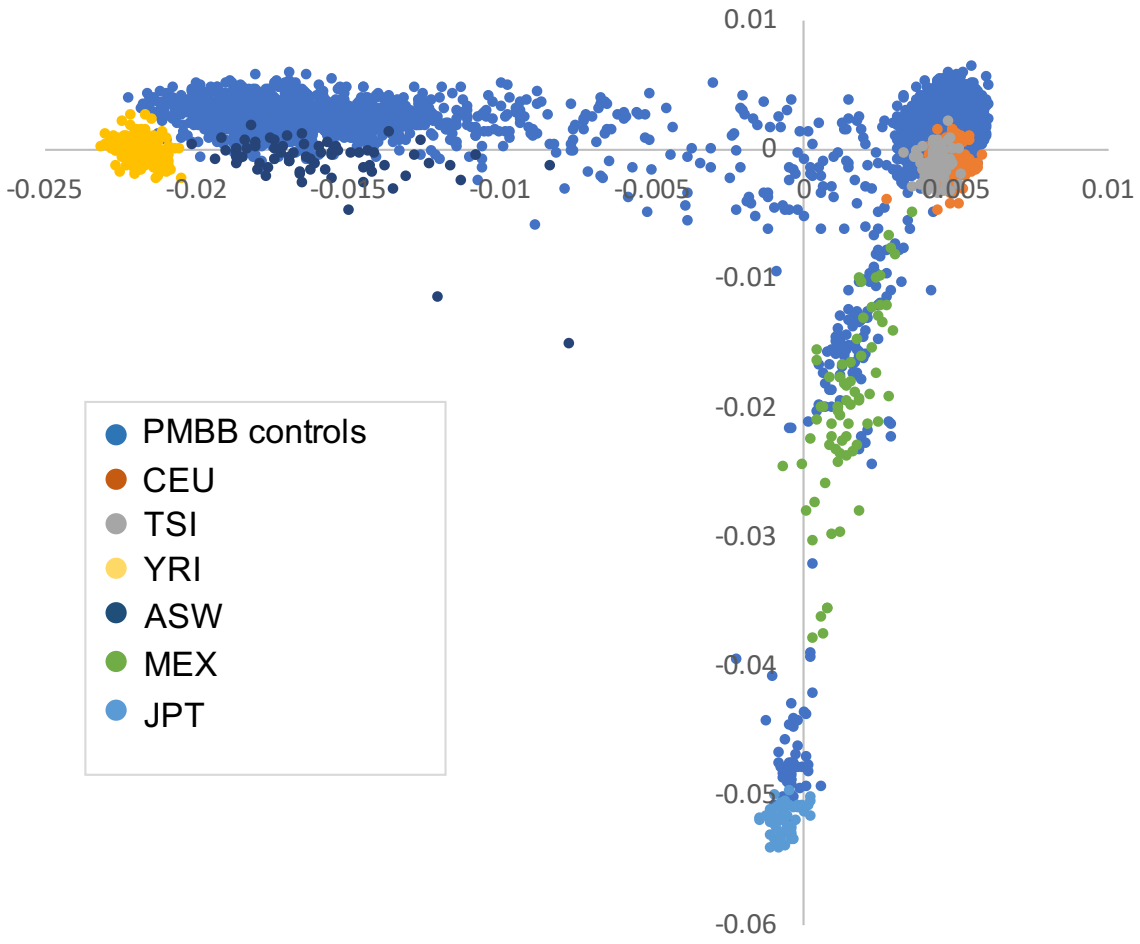
eFigure 2. Ancestry of neuroblastoma cohort inferred from principal component analysis.
Matched germline Illumina SNP array data were utilized to infer ancestry using principal component analysis of pruned SNPs together with the data from 1000 Genomes.



eFigure 3. *BARD1* germline variants validated by Sanger sequencing. A. Lollipop figure depicting pathogenic germline variants in neuroblastoma cases. **B.** Sanger validation of pathogenic germline variants in neuroblastoma. See **eTable 5** for primers used for Sanger sequencing. Variant annotations with respect to ENST00000260947.



eFigure 4. *EZH2* P/LP germline and somatic variants validated by Sanger sequencing. A. Lollipop figure depicting pathogenic germline and somatic variants in neuroblastoma cases. **B.** Sanger validation of pathogenic germline and somatic variants in neuroblastoma patients PAVCJZ, PASTXV, and PASEGA. **C.** Sanger validation of *EZH2* somatic deletion in PASEGA. See **eTable 5** for primers used. Variant annotations with respect to ENST00000320356.



eFigure 5. Ancestry of PMBB cohort inferred from principal component analysis.

IV. BANNER AUTHOR LISTS AND CONTRIBUTIONS

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Contribution: All authors contributed to securing funding, study design and oversight. All authors reviewed the final version of the manuscript.

Patient Recruitment and Regulatory Oversight
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Contributions: JW manage patient recruitment and regulatory oversight of study. AP, AB, KH, YK recruitment and enrollment of study participants.

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Contribution: JW, ML, FV, SD oversight of lab operations. ML, FV, AK, SD, TT, LM perform sample processing. NH, JD are responsible for sample tracking and the laboratory information management system.

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Contribution: All authors contributed to the development and validation of clinical phenotypes used to identify study subjects and (when applicable) controls.

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Contribution: A.V., S.S.V. are responsible for the analysis design and infrastructure needed quality control genotype and exome data. Y.B. performed the analysis. T.D. and A.V. provide variant and gene annotations and their functional interpretation of variants.

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