American Association for Cancer Research

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Rare Pathogenic Germline Variants Were Observed in Sporadic Pediatric Neuroblastoma Cases and Were Inherited

PHILADELPHIA – Pediatric patients with sporadic neuroblastoma harbored rare pathogenic variants in cancer predisposition genes that were inherited and associated with worse disease outcomes, according to data presented during Week 1 of the virtual AACR Annual Meeting 2021, held April 10-15.

Neuroblastoma is a pediatric cancer arising from the sympathetic nervous system. Approximately 1-2 percent of all cases have a positive family history of disease, and are classified as familial neuroblastoma, explained presenting author Emily Blauel, MD, an attending physician at the Children's Hospital of Philadelphia. In these cases, patients are likely to have an underlying pathogenic germline mutation in the *ALK* or *PHOX2B* gene.

The remaining cases, which represent the vast majority, are thought to arise sporadically, Blauel added. While recent studies have estimated that 8-10 percent of pediatric patients spanning all cancer types harbor a rare pathogenic or likely pathogenic germline variant in a cancer predisposition gene, the heritability of such germline variants remains largely unknown due to limited parental data.

"Despite the richness of prior studies, they were unable to definitively determine if the germline variants were inherited due to scarcity of parental germline data," Blauel explained.

With support from the Gabriella Miller Kids First Program (led by John Maris, MD, and Sharon Diskin, PhD), and in collaboration with bioinformatics scientist Zalman Vaksman, PhD, Blauel and colleagues performed whole-genome sequencing of germline DNA from 556 patients with neuroblastoma and one or both of their biological parents. The study included whole genome sequencing (WGS) from 457 patients with both of their biological parents and 99 patients with one of their biological parents. Sequencing of matched tumor DNA and RNA was also performed, including WGS on 336 tumor DNA samples, whole exome sequencing on 326 tumor DNA samples, and RNA sequencing on 207 tumor RNA samples.

The analysis identified 93 pathogenic or likely pathogenic germline variants in known cancer predisposition genes across 90 patients, representing 16 percent of the cohort. Sequencing data from biological parents were available for 85 of these patients, which revealed that 94 percent of the identified germline variants were inherited, with an equal distribution between maternally and paternally inherited patterns. One canonical *ALK* mutation was identified, but no *PHOX2B* mutations were identified.

"This large-scale study allowed us to identify rare pathogenic germline variants and—for the first time—assess whether these variants are inherited or acquired *de novo*," said Blauel. "The results from this study have large implications for patients and their families, and they may aid genetic counseling."

Enrichment of pathogenic or likely pathogenic variants was observed in several known cancer predisposition genes, including *CHEK2*, *BARD1*, and *NSD1*, suggesting that these variants may increase the risk of developing neuroblastoma, Blauel explained. All pathogenic and likely pathogenic germline variants were also observed in the tumor DNA of patients whose tumor sequences were available.

Furthermore, the presence of a pathogenic or likely pathogenic germline variant in a cancer predisposition gene was associated with worse outcomes, including lower odds of 10-year event-free survival (66.9 percent vs. 79.7 percent) and 10-year overall survival (76.5 percent vs. 89.4 percent), compared with those without such variants.

“The observed associations of pathogenic and likely pathogenic germline variants with worse outcomes suggest that we need a more thorough genetic evaluation of patients with neuroblastoma than is currently performed as standard of care,” Blauel noted.

Blauel, Vaksman, and colleagues are currently exploring why parents with pathogenic or likely pathogenic germline variants did not develop neuroblastoma. As this study focused on variants in known cancer predisposition genes, it did not evaluate if pathogenic variants in other genes or in non-coding regions of the genome are associated with neuroblastoma incidence and outcomes. Future plans include extending their analyses genome-wide.

A limitation of the study is that the functional relevance to neuroblastoma is not yet known for many of the pathogenic or likely pathogenic variants identified in this study.

The study was supported by the National Institutes of Health. Blauel declares no conflicts of interest.

Abstract

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Presentation Number:	3030
Publishing Title:	Heritability of cancer predisposition gene mutations in 556 neuroblastoma patients with paired parental DNA whole genome sequences.
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Abstract Body:	**Background:** Neuroblastoma is a childhood cancer that arises from the sympathetic nervous system. Approximately 1% of patients have a family history of disease with causal germline mutations in neuroblastoma predisposition genes, *ALK* or *PHOX2B*. Recent genome-wide association studies (GWAS) of sporadic cases have identified over two dozen susceptibility loci, and next-generation sequencing studies estimate that 8-10% of children with cancer harbor a rare pathogenic (P) or likely pathogenic (LP) germline variant in a cancer predisposition gene (CPG). To date, the heritability and clinical significance of germline P/LP variants remains unknown due to a lack of parental germline DNA. **Methods:** Through the Gabriella Miller Kids First program, we performed whole genome sequencing (WGS) at 30x depth on patient-parent triads (n=457) and dyads (n=99), along with WGS and exome sequencing on matched tumor DNA (n=336) and RNA-sequencing on matched tumor RNA (n=207). Rare variants (allele frequency <0.1% in ExAC, 1000 Genomes, and gnomAD) in a predefined set of CPGs (n=197) were annotated, and pathogenicity was assessed using ClinVar, InterVar, and manual review. Gene-based enrichment was performed by comparison to gnomAD WGS data (n=143,068), excluding TCGA samples. Heritability was evaluated using family-based variant calling. Tumor DNA sequencing was analyzed for second hits or somatic enrichment of the germline P/LP variant. Tumor RNA sequencing was interrogated to identify expressed P/LP variants and preferential allelic expression. Ongoing analyses are utilizing polygenic risk scores from our GWAS study to inform tumor penetrance. **Results:** We observed 78 P/LP germline variants in CPGs in 75 probands (13.5%). Of the 72 probands with trio data, 95% of the P/LP variants were inherited (54% maternal, 46% paternal). Several CPGs showed enrichment of P/LP variants,

including *BARD1* (p=0.001; OR 16.16; 95% CI 3.21-50.45). We observed one canonical *ALK* mutation (R1275Q), but no *PHOX2B* mutations. For patients with corresponding tumor samples, all germline P/LP variants were observed in the tumor DNA. Germline P/LP variants were associated with high-risk disease as defined by risk group (high vs. low/intermediate, p=0.005), INRG stage (stage M vs stage L1/L2/MS, p=0.024), and *MYCN* amplification status (amplified vs not amplified, p=0.016). **Conclusions:** Here we show that approximately 13.5% of patients with neuroblastoma harbor a rare P/LP germline variant in a CPG, and the vast majority of these are inherited. Neuroblastoma patients with P/LP germline variants are more likely to have high-risk disease and several variants suggest potential therapeutic opportunities. Work is ongoing to understand the genetic factors that explain why parents harboring the same P/LP variant did not develop neuroblastoma, and to determine the genetic counseling implications of these data.

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