Biallelic inactivation of \textit{PBRM1} as a molecular driver in a rare pineoblastoma case: illustrative case

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BACKGROUND Pineoblastomas are a rare and aggressive pediatric neuroectodermal tumor subtype. Because of their rarity, pineoblastomas are still poorly understood, and there is little research delineating their molecular development and underlying genetic phenotype. Recent multiomic studies in pineoblastomas and pineal parenchymal tumors identified four clinically and biologically relevant consensus groups driven by signaling/processing pathways; however, molecular level alterations leading to these pathway changes are yet to be discovered, hence the importance of individually profiling every case of this rare tumor type.

OBSERVATIONS The authors present the comprehensive somatic genomic profiling of a patient with pineoblastoma presenting with the loss of protein polybromo-1 (\textit{PBRM1}) as a candidate genomic driver. Loss of \textit{PBRM1}, a tumor suppressor, has been reported as a driver event in various cancer types, including renal cell carcinoma, bladder carcinoma, and meningiomas with papillary features.

LESSONS This is the first report presenting biallelic loss of \textit{PBRM1} as a candidate molecular driver in relation to pineoblastoma.

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KEYWORDS pineoblastoma; pediatric tumor; tumor

Supratentorial primitive neuroectodermal tumors (PNETs) are a subset of rare primary central nervous system (CNS) tumors. Because of their rarity and sparse consensus regarding their histopathological diagnoses, there has not been significant progress made in understanding their underlying genetic and molecular development. Pineoblastomas, which represent approximately half of all pineal parenchymal tumors, are often studied in the context of PNETs. This is because they are classically described as poorly differentiated high-grade tumors with variable reactivity to neuronal and glial histopathological markers. Beyond that, however, the genomic drivers of this disease remain largely unknown. Liu et al. assessed the molecular phenotypes of pineal parenchymal tumors through a global methylation-based classification and correlated the subgroups with distinct copy number landscapes, identifying a subgroup with increased intertumoral heterogeneity. However, molecular mechanisms and genomics drivers leading to these changes in pathways and genomic instabilities that form these classifications remain largely unknown.

Here we report a patient with pineoblastoma presenting with cervical spine lesions. After the intradural extramedullary spinal cord tumor biopsy, whole-exome sequencing (WES) revealed a somatic genetic profile consistent with loss of protein polybromo-1 (\textit{PBRM1}) together with various copy number variations (CNVs), some of which were previously reported in pineoblastomas. Given the rarity of pediatric pineoblastomas, genomic profiling of every case plays an essential role in improving the understanding of molecular mechanisms behind this aggressive tumor type. To our knowledge, this is the first report of a \textit{PBRM1} biallelic loss in a pineoblastoma case.
Illustrative Case
Case Overview
A 6-year-old male presented to the pediatric department with severe headaches and diplopia. On initial magnetic resonance imaging (MRI) of the brain, a 2 × 1.5-cm pineal mass was noted (Fig. 1A and B). Complete neuraxis MRI was obtained. This showed a heterogeneously enhancing lesion within the pineal gland concerning for pineoblastoma. Also noted were several leptomeningeal enhancing lesions within the cervical spine, suggestive of craniospinal dissemination secondary to the primary pineal lesion. Because there was evidence of dissemination, and because of the suboptimal location of the lesion, surgical excision and biopsy of the primary lesion were not pursued. Instead, C4 and because of the suboptimal location of the lesion, surgical excision was performed with the patient under general anesthesia, with intradural extradural spinal cord tumor biopsy. The tumor was sent for pathology, and preliminary results suggested a small, round blue cell tumor with high-grade features. It was noted during the surgical biopsy that the patient had elevated intracranial pressure because there was significant cerebrospinal fluid flow in the surgical field.

Histopathology revealed high-grade neuroepithelial neoplasm, most consistent with pineoblastoma, World Health Organization (WHO) grade IV. Specifically, histopathological examination showed a hypercellular tumor with uniform proliferation of so-called small blue cells with cytological features similar to other PNETs: round or carrot-shaped and hyperchromatic nuclei with nuclear molding and scant cytoplasm. Mitotic activity was noted to be brisk, exceeding 15 mitotic figures per 10 high-power fields. Numerous atypical mitoses and apoptotic bodies were seen. Focally, the tumor cells showed vague Homer Wright pseudorosettes with tumor cells surrounding the neuropil. Reticulin stain highlighted scattered fragmented reticulin fibers. Immunohistochemical studies showed strong antisynaptophysin immunolabeling, and tumor cells were negative for chromogranin, NeuN, and glial fibrillary acidic protein. The Ki-67 proliferation index was high, approaching 35%–40%. There were rare tumor-infiltrating CD45+ or CD3+ immune cells. Histological features and the immunophenotype of tumor cells in the current biopsy are most consistent with metastatic high-grade neuroepithelial neoplasm consistent with pineoblastoma, WHO grade IV (Fig. 2). The patient underwent radiation therapy simulation after the biopsy. He was concurrently given chemotherapy as per ACNS0332. The patient currently continues to be seen in follow-up in an outpatient setting for treatment and management.

Genomic Findings
WES of the spinal lesion was performed with matching normal blood in order to detect somatic single-nucleotide variations (SNVs), insertions/deletions (INDELs), and CNVs (Fig. 3). Sequencing was performed at the Yale Center for Genome Analysis using the Illumina NovaSeq 6000 system with 2 × 101–bp reads following the capture of the regions using IDT xGen Exome Research Panel version 1 (Integrated DNA Technologies, Coralville, IA). Mean coverage of 151.3 × 230.6 × was achieved for normal and tumor, respectively. CNV analysis and generation of plots were performed using GATK Best Practices guidelines (GATK4.1.9.0) with some modifications; specifically, the number of smoothing iterations per fit was set to 1 from the default recommended setting of 0, and the number of change points penalty factor was set to 5 from the default recommended value of 1. Somatic variant calling for SNVs/INDELs together with annotation was performed as previously described in reports from our institution.

Somatic WES analysis revealed 26 SNVs/INDELs. Prioritization of these genes was performed based on known cancer association, which identified four SNVs/INDELs. These four events were further prioritized by their variant allele frequencies, with the most significant being a PBRM1 inframe deletion (NM_004958: p.787_789del) on one of its bromodomains (BRDs) (BD6), with an allele frequency of 94.9%. Also worth mentioning is that even though a mechanistic target of rapamycin missense mutation (NM_004958: p.A677Vp) was identified, it was not an activating mutation, leaving the biallelic loss of PBRM1, a tumor suppressor gene, as the single most significant candidate driver of our case. Somatic CNV analyses revealed that 7.0% of the genome had amplifications, and 13% displayed deletions, whereas 24.2% of the genome had loss of heterozygosity (LOH). Analysis of the somatic CNV/LOH events revealed deletions on chromosomes 3p21, 3p14, 6, 11, 14, and 22. Furthermore, integrative analysis of the somatic SNV/INDEL and CNV data revealed that focal deletion on chr3p21 overlapped with the PBRM1 locus, leading to a potentially biallelic loss combined with the deleterious inframe deletion. Indeed, the variant allele frequency of the inframe deletion was identified as 94.9%, confirming the deleted copy of PBRM1 was the wild-type copy. Also worth noting is that the inframe deletion on the remaining copy is a very rare variant with an allele frequency <0.001 in the gnomAD database (version 2.1.1). The location of the inframe deletion event (787_789del) overlaps with the BD6 of PBRM1.

Discussion
Observations
Pineoblastomas are a high-grade, rare, and understudied embryonal brain tumor type with a poor overall survival rate. Pineoblastomas are difficult to differentiate from other types of PNETs clinically due to their variable reactivity to neuronal and glial histopathologic
markers, and therefore they are often studied under the context of PNETs. Advances in molecular characterization of neuroectodermal tumors have aided the ability to more accurately identify molecular processes leading to this rare tumor type. Patients with germline mutations in RB1 and DICER1 have been shown to have increased risk of developing pineoblastoma. Snuderl et al. further identified mutations in DROSHA (upstream of DICER1) and PDE4DIP (comprising the DUF1120 protein domain), present exclusively in

![Image of H&E staining](image1)

**FIG. 2.** A and B: Hematoxylin and eosin staining reveals a PNET, showing a solid growth of densely packed tumor cells with irregular hyperchromatic nuclei, scant cytoplasm, nuclear molding, atypical mitotic figures (red arrows), karyorrhectic nuclei (black arrow), and rosette formation (green arrows). Focally, the tumor cells show crush artifact (white arrow). Original magnification × 100 (A) and × 400 (B). C: The tumor shows strong expression of synaptophysin (marker of neuronal differentiation). Original magnification × 100. D: Expression of glial fibrillary acidic protein, an astrocytic antigen, is not detected in the tumor. Original magnification × 100. E: A reticulin stain highlights scattered fragmented reticulin fibers. Original magnification × 100. F: The proliferative index (Ki-67) is high. Original magnification × 100. G: Nuclear expression of INI1/BAF47 is retained. Original magnification × 100. H: Neoplastic cells show normal nuclear staining for H3K27me3, which is consistent with absence of aberrant loss of methylation. Original magnification × 100.

![Image of copy number alteration](image2)

**FIG. 3.** A: Deletion events in blue frames and amplification events in red frames. B: LOH is depicted in green frames. Tumor is compared with matched blood from the patient.
pineoblastoma. Recently, a study on a large cohort of patients with pineoblastoma and supratentorial PNET, which integrated global DNA methylation profiling, copy number analysis, WES, and targeted sequencing analyses, revealed five molecular subgroups with distinct copy number profiles and miRNA biogenesis defects, RB1 loss, and MYC activation. Notably, chromosome 14 loss was reported in one of the consensus groups, which overlaps with the CNV findings in our case. However, individual molecular mechanisms underlying the development of the disease remain to be explored.

Our integrative genomic analysis remarkably revealed an inframe deletion on PBRM1 (NM_001350075:p.787_789del), combined with a focal deletion and LOH on chr3p21, overlapping with the PBRM1 locus. Indeed, the variant allele frequency of the inframe deletion was identified as 94.9%, confirming that the deleted copy was the wild-type copy. PBRM1 has been described to play a role in cellular apoptosis and stress response, such that it indirectly decreases reactive oxygen species (ROS) and supports cellular viability. Loss of PBRM1 appears to drive cell growth and genomic instability, and alterations of PBRM1 have been previously described in clear cell renal cell carcinoma (RCC), as well as in a subset of papillary RCC, bladder carcinoma, and meningioma with papillary features. Furthermore, PBRM1 encodes BRG1-associated factor 180 (BAF-180), a component of the multiprotein polybromo- and BRG1-associated factor-containing complex switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex. Mutations affecting the SWI/SNF complex have also been linked to multiple cancers, including central nervous system neoplasms such as schwannomas and clear cell meningiomas.

Interestingly, the inframe deletion event of PBRM1 corresponds to its sixth BRD. BRDs are evolutionarily conserved protein–protein interaction modules that are found in a wide range of proteins with diverse catalytic and scaffolding functions and are present in most tissues. BRDs selectively recognize and bind to acetylated Lys residues—particularly in histones—and thereby have important roles in the regulation of gene expression. Furthermore, it was reported that PBRM1 acts as a p53 lysine-acetylation reader to suppress renal tumor growth through its fourth BRD (BD4).

Also, from a translational standpoint, immune therapies are dependent on infiltrating immune tumor responses. In the same way that RCCs with loss of PRB1M have low immunogenicity, we find that the presented pineoblastoma case has low immunogenicity, with rare infiltrating CD45 or CD3 cells on biopsy.

Lessons
Although the previous studies and findings mentioned in this paper all point to a pineoblastoma subgroup with increased genomic instability, the link between the molecular mechanism driving the tumor formation and the observed genomic instability seems to be missing. Furthermore, although PBRM1 was reported as a tumor driver in various cancer studies, it has not been reported in a pineoblastoma case before. Therefore, this implies the significance of the presented case as the first report of a biallelic PBRM1 loss, potentially acting as the driver for tumor formation and genomic instability in a pineoblastoma case. Further studies will be required to study the frequency and role of loss of PBRM1 in pineoblastomas and its genomic and clinical implications, but this study shows the importance of individually profiling every case, both from a translational standpoint to benefit individual patients and also to understand the underlying molecular drivers of this rare and aggressive tumor type.

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Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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Conception and design: Antonios, DiLuna, Erson-Ömey. Acquisition of data: Antonios, Yalcin, Darbinyan, Hong, DiLuna, Erson-Ömey. Analysis and interpretation of data: Antonios, Yalcin, Darbinyan, Hong, Erson-Ömey. Drafting the article: Antonios, Yalcin, Koo, Hong, DiLuna, Erson-Ömey. Critically revising the article: Antonios, Darbinyan, Koo, Hong, DiLuna, Erson-Ömey. Reviewed submitted version of manuscript: Antonios, Darbinyan, Hong, DiLuna, Erson-Ömey. Approved the final version of the manuscript on behalf of all authors: Antonios. Statistical analysis: Antonios, Yalcin. Administrative/technical/material support: Antonios, DiLuna. Study supervision: Antonios, DiLuna, Erson-Ömey.

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