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Posted Date: 29 July 2024

doi: 10.20944/preprints202407.2300.v1

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Article

Implementation of Methylation Profiling of Central Nervous System Tumors at Largest Public Health Center in Brazil

Beatriz Martins Wolff ¹, Yuri Casal ², Lucas Liro Vieira ¹, Gleyson Francisco da Silva Carvalho ¹, Mariana Ribeiro ¹, Rafaela Mendes ¹, Maria Fernanda Freire Chagas ¹, Lissandro Rolim ¹, Yanca Gasparini ¹, Eder Alencar Moura ¹, Felipe D Almeida Costa ³ and Leslie Domenici Kulikowski ¹

¹ Cytogenomics Laboratory, Department of Pathology, Faculty of Medicine, University of São Paulo, São Paulo, Brazil.

² Department of Pathology, Hospital das Clínicas, University of São Paulo, São Paulo, Brazil.

³ Department of Anatomic Pathology, AC Camargo Cancer Center, São Paulo, Brazil.

* Correspondence: beatriz.wolff@usp.br

Abstract: Tumor entities of the Central Nervous System (CNS) are defined by the WHO classification and range from benign neoplasms to highly malignant tumors, making histopathological classification challenging. This diverse spectrum complicates clinical decision-making and the interpretation and validation of clinical trial results. The methodology based on DNA methylation profiling of CNS tumors offers greater diagnostic precision compared to traditional morphological methods. In this study, we analyzed 16 DNA samples from medulloblastomas and ependymomas, obtained from paraffin-embedded blocks from the Pathology Division archives of HCFMUSP, and with prior histological analysis. The samples were processed using the Infinium MethylationEPIC BeadChip (Illumina®) and methylation data analysis tools in R Studio. The data were submitted to the MolecularNeuropathology.org platform for methylation-based classification and CNV chart generation. The classifier was consistent with the previous diagnosis in all cases, refining the diagnosis in 54% of cases to subclass, 38% to subtype, and 8% to family. The platform also provided CNV estimates for 87% of cases, revealing crucial information on genetic alterations such as gains, losses, amplifications, and gene fusions. Despite the challenges of implementation within the Brazilian Public Health System (SUS), the results demonstrate the method's robustness and reproducibility, promising invaluable support for precise diagnoses and the development of more effective therapeutic strategies.

Keywords: cancer; methylation; array; medulloblastoma; ependymoma

1. Introduction

Considerations on Central Nervous System Tumors

The classification of CNS (Central Nervous System) tumors is defined according to WHO (World Health Organization) criteria, which since the 4th edition published in 2016, uses molecular parameters in addition to the histopathological ones already employed for diagnosing the wide range of tumor entities [1,2].

These tumors are clinically and biologically disparate, encompassing a broad spectrum from benign neoplasms to highly malignant tumors. Benign tumors (WHO CNS grade 1) are mostly characterized by low mitotic rate, cellular uniformity, and slow growth, and often can be treated surgically. Malignant tumors vary in prognostic grades (WHO CNS grades 2-4) and are characterized by rapid and uncoordinated proliferation, being additionally stratified based on mitotic index and presence of necrosis or microvascular proliferation. They are generally treated with a combination of radiotherapy and chemotherapy [2-4].

The main categories of CNS tumors are Gliomas, encompassing astrocytomas, oligodendrogliomas, and ependymomas; Glioneuronal and neuronal tumors such as gangliogliomas; Embryonal tumors such as medulloblastomas; Meningiomas; and others (Table 1) [2,5,6].

Table 1. Categories of Central Nervous System Tumors according to the 5th edition of the World Health Organization.

Categories of Central Nervous System Tumors (WHO 5th edition)
Gliomas, glioneuronal and neuronal tumors
Choroid plexus tumors
Embryonal tumors
Pineal tumors
Cranial and paraspinal nerve tumors
Meningioma
Non-meningothelial mesenchymal tumors involving the CNS
Melanocytic tumors
Hematolymphoid tumors involving the CNS
Germ cell tumors
Sellar region tumors
Metastases to the CNS

All categories have subcategories and specific criteria that must be strictly met to assign a tumor to a given group, making diagnosis challenging in rare or atypical cases. Previous studies have reported interobserver variability in the histopathological diagnosis of many CNS tumors, with diffuse gliomas, ependymomas, and embryonal tumors standing out [2–4].

Studies demonstrate that methylation profiling offers greater diagnostic precision compared to traditional methods, allowing for detailed stratification of tumor subclasses and providing critical information on copy number variations (CNV) and other genetic alterations. This level of detail is essential for developing more effective and personalized therapeutic strategies. The correlation of previous histological results with methylation-based classification will be conducted using the MolecularNeuropathology.org platform to obtain accurate classification and compare it with previously performed histological diagnoses [7,8].

This study aimed to implement and standardize the technique for constructing DNA methylation profiles of Central Nervous System (CNS) tumors in the Cytogenomics laboratory at the Hospital das Clínicas, Faculty of Medicine, University of São Paulo, evaluating the DNA methylation profile in 16 samples of CNS tumors, specifically medulloblastomas and ependymomas.

The implementation of this technique within the context of the Brazilian Public Health System (SUS), despite being laborious and costly, promises great benefits. The ability to refine the diagnosis and provide a detailed molecular profile of CNS tumors is invaluable for clinical practice. Moreover, the robustness and reproducibility of the results highlight the potential of the methylation methodology to become a standard in the diagnosis of CNS tumors, significantly improving patient prognosis and quality of life.

Results

Methodology Implementation

The classification of CNS tumors based on methylation profiling was performed on 16 retrospective cases encompassing two tumor entities: medulloblastomas and ependymomas, including 9 and 7 samples, respectively. More clinical and histopathological information is detailed in Table 2.

Table 2. The table contains the identification of the samples (ID), the histopathological diagnosis (previous diagnosis), the patient's sex (M) Male and (F) Female, the age at diagnosis, the location of the tumor and the classes of methylation divided according to the Classifier version. It also presents the correspondence with the platform (match) and the score obtained (score), in addition to the methylation status of the *MGMT* gene promoter. The classification is only valid with scores ≥ 0.9 .

Histopathological Diagnosis		Sex	Age at Diagnosis	Topography	brain_classifier_v11b4_sample_report			brain_classifier_v12.5_sample_report			
ID	Previous Diagnosis				Methylation Class	Match	Score	Methylation Class	Match	Score	MGMT status
B2	Classic medulloblastoma	M	11	Cerebellum	Methylation class family Medulloblastoma group 3 and 4	yes	0.99	Medulloblastoma Medulloblastoma no-WNT/no-SHH activated Medulloblastoma Grup 4 MC Medulloblastoma, no-WNT/no-SHH, Grup 4 subtype, subclass VIII	yes	0.99	Not Methylated
B3	Classic medulloblastoma	M	2	Cerebellum	Methylation class family Medulloblastoma group 3 and 4	yes	0.99	Medulloblastoma Medulloblastoma no-WNT/no-SHH activated Medulloblastoma Grup 3 MC Medulloblastoma, no-WNT/no-SHH, Grup 3 subtype, subclass IV	yes	0.99	Not Methylated
B4	Desmoplastic/nodular medulloblastoma	M	14	Cerebellum	Methylation class family Medulloblastoma group 3 and 4	yes	0.99	Medulloblastoma Medulloblastoma no-WNT/no-SHH activated Medulloblastoma Grup 4 MC Medulloblastoma, no-WNT/no-SHH, Grup 4 subtype, subclass VII	yes	0.99	Not Methylated
B5	Desmoplastic/nodular medulloblastoma	M	1	Cerebellum	Methylation class family Medulloblastoma, SHH	no	0.83	Medulloblastoma Medulloblastoma, SHH-activated Medulloblastoma, SHH-activated, subtype 2 MC Medulloblastoma, SHH-activated, subclass 2	yes	0.99	Not Methylated
B6	Medulloblastoma with extensive nodularity	M	16	Cerebellum	Plexus Tumor family of methylation classes	no	0.51	Medulloblastoma	no	0.75	Methylated
B7	Classic medulloblastoma	M	6	Cerebellum	Medulloblastoma methylation class family group 3 and 4	yes	0.99	Medulloblastoma Medulloblastoma no-WNT/no-SHH activated Medulloblastoma Grup 3 MC Medulloblastoma, no-WNT/no-SHH, Grup 3 subtype, subclass II	yes	0.99	Not Methylated
B8	Desmoplastic/nodular medulloblastoma	F	2	Cerebellum	Medulloblastoma methylation class family, SHH	yes	0.99	Medulloblastoma Medulloblastoma, SHH-activated Medulloblastoma, SHH-activated, subtype 1	yes	0.99	Not Methylated
B9	Classic medulloblastoma	M	22	Cerebellum	Methylation class family Medulloblastoma group 3 and 4	yes	0.99	Medulloblastoma Medulloblastoma no-WNT/no-SHH activated Medulloblastoma Grup 4 MC Medulloblastoma, no-WNT/no-SHH, Grup 4 subtype, subclass VII	yes	0.99	Not Methylated
B10	Anaplastic medulloblastoma	M	6	Posterior fossa	Plexus Tumor family of methylation classes	no	0.43	Medulloblastoma Medulloblastoma no-WNT/no-SHH activated Medulloblastoma Grup 4 MC Medulloblastoma, no-WNT/no-SHH, Grup 4 subtype, subclass V	yes	0.98	Not Methylated
B11	Ependymoma	M	2	Posterior fossa	Plexus Tumor family of methylation classes	no	0.42	Ependymal tumors Ependymal tumors	no	0.37	Not Methylated
B12	Ependymoma	M	1	IV Ventricle				Posterior fossa ependymoma group A Posterior fossa ependymoma group A1 MC Posterior fossa group A (PFA) ependymoma, subclass 1a (novel)	yes	0.90	Not Methylated
B13	Ependymoma	F	4	Cerebellum	Methylation class ependymoma, posterior fossa group A	yes	0.97	Ependymal tumors Posterior fossa ependymoma group A Posterior fossa ependymoma group A1 MC Posterior fossa group A (PFA) ependymoma, subclass 1b	yes	0.99	Not Methylated
B14	Ependymoma	F	7	Posterior fossa	Methylation class ependymoma, posterior fossa group A	no	0.83	Ependymal tumors Posterior fossa ependymoma group A Posterior fossa ependymoma group A1 MC Posterior fossa group A (PFA) ependymoma, subclass 1b	yes	0.99	Not Methylated
B15	Ependymoma	F	38	Posterior fossa				Ependymal tumors Ependymal tumors	no	0.62	Not Methylated
B16	Ependymoma	F	16	IV Ventricle	Methylation class ependymoma, posterior fossa group B	no	0.30	Posterior fossa ependymoma group B and posterior fossa subependymoma Posterior fossa ependymoma group B MC Posterior fossa group B (PFB) ependymoma, subclass 3 (novel)	yes	0.98	Methylated
B17	Ependymoma	F	7	Posterior fossa	Methylation class subependymoma, spinal	no	0.73	Ependymal tumors Spinal subependymoma Spinal subependymoma [subtype A]	yes	0.99	Methylated

The first step for implementation was the extraction of DNA from FFPE tissues. Tissue resection is subject to material evaluation by a certified pathologist, the tumor region was identified, and its content was confirmed to be above 60%. Accordingly, we standardized the use of 5 sections of 10 μm thickness from tissue fixed in 10% buffered formalin for DNA extraction. The concentrations obtained ranged from 10.5 to 478 $\text{ng}/\mu\text{l}$, with an average of 166 $\text{ng}/\mu\text{l}$ and a median of 92.6 $\text{ng}/\mu\text{l}$. The DNA input was standardized at 250 ng for the bisulfite conversion assay, and all samples had sufficient quantification for the methylome assay.

Comparison between Previous Diagnosis and Epigenetic Classification

We compared the classifier score results with histopathological findings according to the recommendations of Capper et al [3,4]. A match with a score above 0.9 was obtained in 13/16 cases (81%) in at least one version of the classifier.

In total, 7/16 cases (44%) were classified in both versions (v11b4 and v12.5), including one ependymoma and six medulloblastomas; 6/16 cases (37%) matched only in the most recent version (v12.5), including four ependymomas and two medulloblastomas, and 3/16 cases (19%), composed of two ependymomas and one medulloblastoma, did not achieve a sufficient score for classification (<0.9).

The classifier result was consistent with the initial diagnosis in all classified cases (whether adequately scored or not).

Of the 13 classified cases, the subclass was defined in seven (54%), five cases (38%) were stratified to the subtype, and one (8%) to the family. Of the 16 samples analyzed, 13 did not have a methylated MGMT gene promoter (Table 2).

Evaluation of Copy Number Variation (CNV) Data

The platform also offers estimates of copy number gains and losses, gene amplification, and fusions. We investigated the CNV profiles for each case and were able to obtain informative graphs in 87% of them. In two samples that did not achieve a classification score (B6 and B11), the graphs showed scattered probes and were unreliable for the intended analysis, while the last case (B15) presented a clean and noise-free graph, allowing the identification of 1p deletion, deletions of chromosomes 3, 6, 13, 14, 17, and 22, and the C19MC gene (Figure 1).

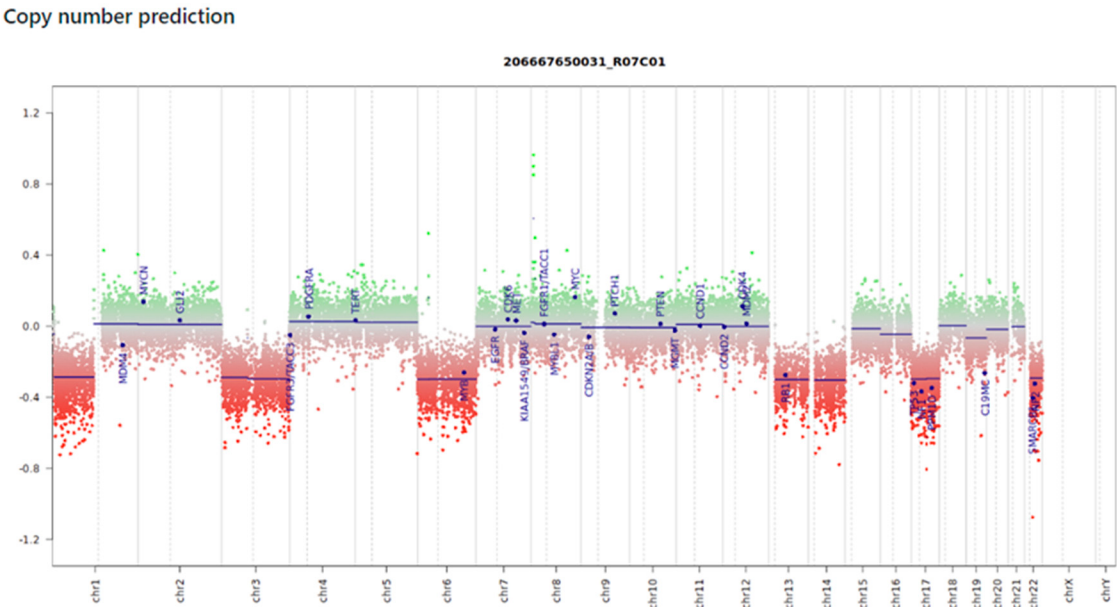


Figure 1. CNVs graph provided by the Classifier showing deletion of 1p, deletion of chromosomes 3, 6, 13, 14, 17 and 22 and the C19MC gene in case B15.

Additional Analyses

We also performed additional bioinformatics analyses. From a dissimilarity matrix, we conducted a principal coordinates analysis (PCoA) using the top 1,000 differentially methylated probes between medulloblastomas and ependymomas. The two tumor entities were linearly separable and highly dissimilar, with homogeneous aggregation by group. Samples B6, B11, and B15 did not achieve the necessary score for classification using the Heidelberg Classifier; however, the

segregation of B11 and B15 with other ependymomas can be observed (Figure 2). Sample B6 did not present sufficient quality for additional analysis.

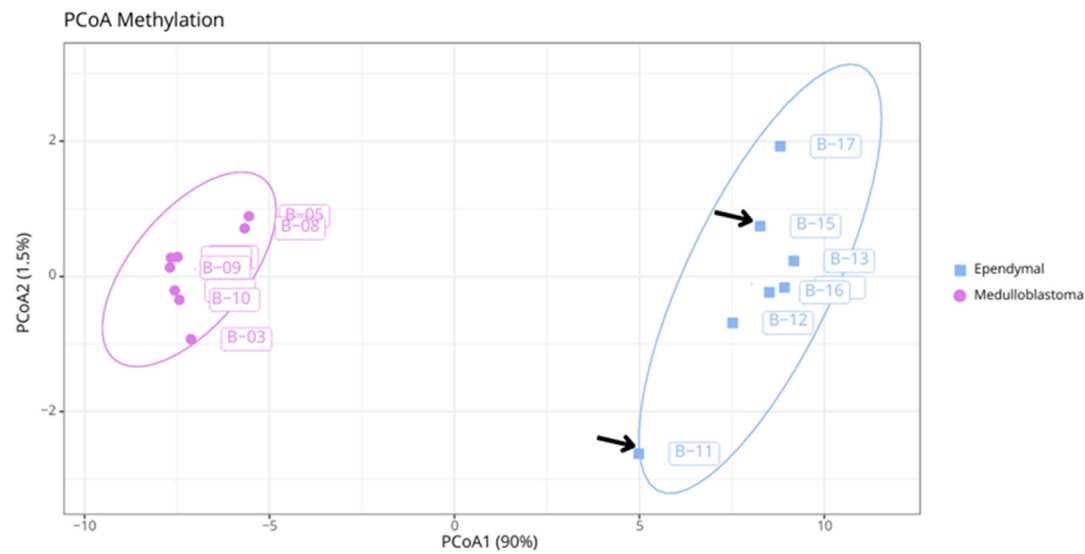


Figure 2. PCoA shows high dissimilarity between groups and homogeneous grouping by tumor entity based on methylation profile. Medulloblastomas are represented by lilac colored spheres while ependymomas appear like blue squares. Samples B11 and B15 highlighted by arrows appear associated with other ependymomas while B6 did not present sufficient quality for this additional analysis.

In an attempt to clarify the unclassified cases, we submitted the raw data to EpiDiP for UMAP mapping with thousands of already known tumor entities. Case B6 did not approach any group, while B11 and B15 approximated the ependymoma group, as shown in Figure 3.



Figure 3. Image of the EpiDiP platform based on the tumor DNA methylation profile. Medulloblastoma (B6) does not joined any group, but ependymomas (B11 and B15) are close to other groups of ependymomas, highlighted on the graph by a purple circle. The medulloblastomas and ependymomas included in the graph are shown on the right stratified by subtype and location.

Integration of Results

Finally, it was possible to confirm and refine the initial diagnosis in 13/16 proposed cases (81%). Three tumors (19%) did not achieve the necessary score for classification but were correctly identified regarding the represented entities (Table 1). Details on the classifications are represented in the alluvial diagram (Figure 4).

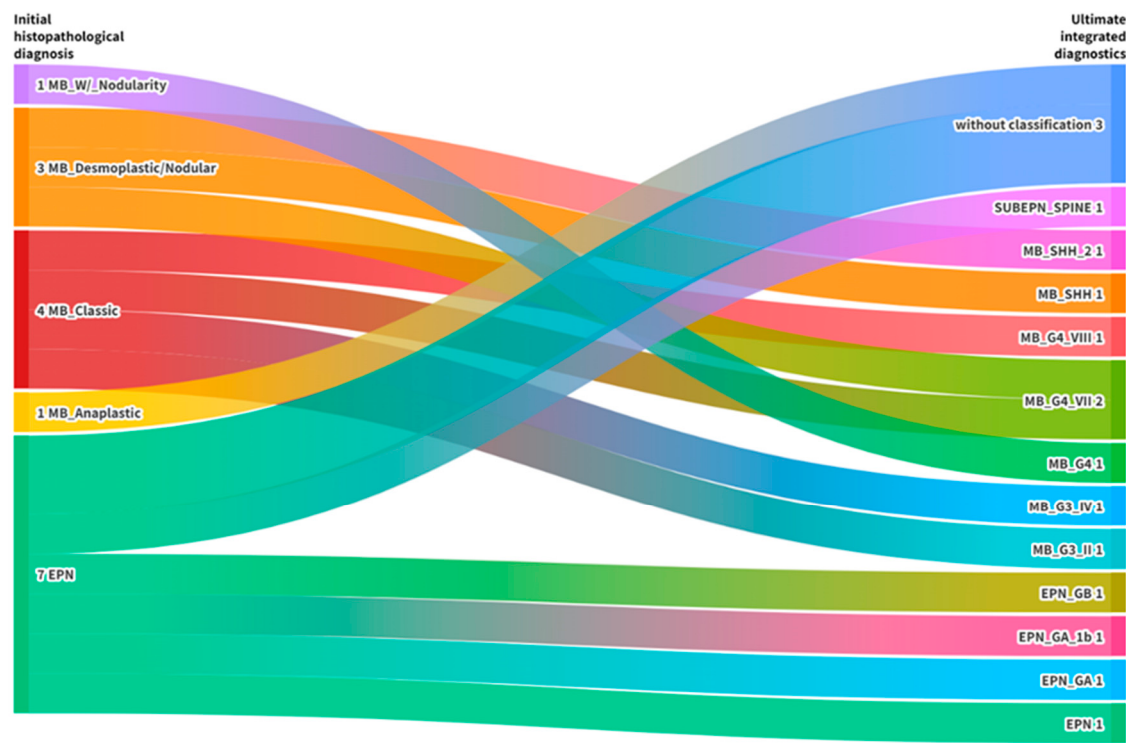


Figure 4. Integration of array data into initial histopathological diagnosis (left) helped establish the diagnosis final integrated (right) in 13 of the 16 cases studied.

Discussion

CNS tumors is invaluable for clinical practice receives patients with suspected CNS tumors based on imaging diagnosis.

After biopsy, all tumors are subject to histopathological evaluation, and some, such as medulloblastomas and ependymomas, to immunohistochemistry. This information composes the histopathological report, released by the pathologist within 15 days from the date of material entry. Access to molecular techniques such as FISH (Fluorescence in situ hybridization) and massive parallel sequencing is extremely rare, and therapeutic approaches are subject to the limited scenario.

DNA methylation profiling significantly contributed to refining CNS tumor diagnoses, adding depth to their classification. This tool has become essential for diagnosing atypical cases in various reference centers worldwide. Pediatric tumors, such as medulloblastomas and ependymomas, are laboriously classified through conventional histopathology, still being subject to interobserver bias [3,4,9,10]. Our study demonstrated the method’s ability to refine diagnosis and provide more information about the molecular profile of CNS tumors from samples with different DNA qualities.

Challenges in Implementation of the Methodology

The implementation of the methodology involved the use of five different commercial kits, in addition to the EPIC array assay, in a continuous workflow of at least 11 days for the total processing of one or more samples. Moreover, obtaining satisfactory amounts of DNA from FFPE samples is a challenge for clinical laboratories.

Thus, the use of CNS tumor methylation profiling within the SUS scope in the current public health scenario would be better employed in challenging cases without consensus on diagnosis, presenting unusual characteristics or conflicting morphomolecular data [9,11].

Methylation Profiling as a Diagnostic tool

The classifier was able to confirm and refine 81% of the proposed retrospective cases, stratifying them to the subclass level, showing that DNA methylation profiling is more refined than the classification proposed by WHO [3,4]. All reports made available by the platform were presented to a pathologist from the pathology department at HCFMUSP for evaluation, and none were refuted.

Although three cases (19% of the cohort) did not achieve sufficient scores for precise classification in the classifier (ranging from 0.37 to 0.75), their tumor entity was correctly indicated by the most recent version (v12.5), albeit with a reliability degree lower than indicated. In this scenario, it is possible to accept the classification as an indication of the clinical diagnosis, in addition to CNVs, amplifications, fusions, and sequencing results, when available [3,4,11]. All classification information is available in Table 2 and Figure 4.

Medulloblastomas

The three samples that did not achieve reliable classification, including one medulloblastoma (B6) and two ependymomas (B11 and B15), were subjected to additional analyses for possible clarification. Case B6 was excluded in the quality control stage of additional analyses; however, we submitted the sample to the classifier to evaluate the platform's performance in different scenarios.

Despite having adequate quantification (85.4 ng/μl), an electrophoresis run after the experiment revealed a high degree of fragmentation of the extracted DNA. This may be directly related to the material collection time (2014), storage, and reagents used for embedding. All these aspects can directly interfere with the sample quality, leading to suboptimal classifier performance [3,4]. The resulting CNV graph from the classification was not informative; however, version 12.5 of the classifier was able to correctly interpret the sample as a medulloblastoma, with a score of 0.75 (Table 2).

While histopathology divides medulloblastomas into three subgroups (WNT, SHH, and Non-WNT/Non-SHH), the methylome separates them into four clinically relevant subgroups (WNT, SHH, Group 3, and Group 4), and can still be stratified into several subclasses [12,13].

Ependymomas

Cases B11 and B15 were within the stipulated quality parameters. Sample B11, embedded in 2018, was slightly more degraded than B15, embedded in 2022. This discrepancy was also seen in the CNV analysis. While B11 presented a noisy and difficult-to-interpret graph, B15 presented a clean graph with clearly delineated gains and losses (Figure 1) that could be used by the pathologist for case understanding.

Investigating CNVs can highlight alterations that assist the pathologist in establishing diagnoses that provide prognostic information or could be of interest for precision medicine [9]. According to Capper et al [3,4], the final diagnostic interpretation can be independently influenced by CNV analysis results and methylation classifier results, as both are considered independent of each other.

During the dissimilarity matrix analysis, it was observed that B11 and B15 grouped with the other ependymomas (Figure 2), demonstrating the robustness of the methylation profile as a classification method [3,4,9]. However, to verify whether this was a spurious association or a real grouping, the cases were subjected to UMAP analysis using EPiDiP. This revealed the proximity of these cases to other known ependymomas (Figure 3). Similar to B6, the classification score for these ependymomas was too low and not considered as valid. Nevertheless, version 12.5 of the classifier was able to identify them as ependymal tumors.

It is important to note that methylation analysis in ependymal tumors results in a complete overhaul of the classification system. This approach eliminates the need for additional classifications

since the individual methylation categories can already identify more homogeneous patient groups in terms of prognosis compared to the previous WHO classification. A notable feature of ependymomas is the observation that tumors in different locations appear to represent distinct entities, despite having similar histology [3,4].

Even though advances in the ependymoma methylome are evident, there are still aspects to be clarified. According to Capper et al. [4], some tumors with morphology suggestive of ependymoma did not correspond to any ependymal methylation class in the classifier. This likely indicates the existence of other classes with histological characteristics of ependymomas that have not yet been defined, which could explain the unclassifiable cases in our cohort.

The Classifier

The Classifier is subject to continuous updates, recognizing more tumor entities with each new version. This evolution is promising, as our understanding of new classes and entities advances, the scope of the Classifier expands concomitantly [3,4]. Thus, we can say that the broader classification potential of version 12.5 is empirically related to deep learning training and improved quality controls, making it more stringent.

Challenges in SUS routine diagnostics

The results demonstrate that DNA methylation profiling is valuable for the clinical diagnosis of CNS tumors in the Brazilian public health system. However, the high costs and extended processing time associated with methylation arrays pose challenges for their routine use in Brazil. These factors limit their adoption, necessitating further evaluation and consideration before widespread implementation in the SUS framework.

Materials and Methods

Case Selection

All 16 FFPE blocks were obtained from the archives of the Division of Pathology at the Hospital das Clínicas, Faculty of Medicine, University of São Paulo, and correspond to CNS tumors from patients treated at the hospital. The samples included cases of medulloblastoma and ependymoma, whose molecular subclassification has diagnostic, prognostic, and therapeutic relevance. We excluded cases without a prior diagnosis for comparison and samples outside quality parameters. The project was approved by the Ethics Committee for Research Project Analysis of the Clinical Directorate of Hospital das Clínicas, Faculty of Medicine, University of São Paulo (Approval Number: 5.537.550).

DNA Extraction

Extraction was performed using the QIAamp® DNA FFPE Advanced Kit. The procedure removes paraffin and reverses formalin crosslinks in the DNA sample. Additionally, the UNG enzyme (Uracil-N-Glycosylase) specifically removes uracils resulting from cytosine deamination, a common event in FFPE samples. The extraction protocol was optimized by introducing a 16-hour pre-processing incubation at 56°C in a deparaffinization solution containing ultrapure water, FTB buffer, and proteinase K. The newly extracted DNA was quantified using the Qubit 4.0 Fluorometer (Invitrogen®).

Epigenomic Arrays

Bisulfite conversion was performed using the EZ DNA Methylation Kit (Zymo Research®) according to the manufacturer's protocol, with the alternative cycling suggested for methylation array assays (95°C for 30 seconds, 50°C for 60 minutes) x 16 cycles. After conversion, the samples underwent a pre-array restoration step as indicated by the manufacturer, Illumina. The process used the Infinium HD FFPE Restore Kit (Illumina®) and the ZR-96 Genomic DNA Clean & Concentrator

Kit (Zymo Research®). Together, they perform an isothermal PCR restoring the genomic regions covered by the BeadChip array and of clinical interest. The DNA was processed using the Illumina Infinium HumanMethylation EPIC BeadChip array (Illumina®) according to the manufacturer's instructions. The BeadChip was scanned by iScan, generating raw data in .idat format.

Data Analysis

The raw data files generated after scanning (.idat) were submitted to the online DNA methylation classifier for CNS tumors at <https://www.molecularneuropathology.org>. A report was generated for each tumor in versions v11b4 (created in 2017) and v12.5 (created in 2022), providing prediction scores for methylation classes and CNV estimation graphs. All samples were compared with histopathological findings, as proposed by Capper and colleagues in 2018 [4].

In parallel, we conducted additional analyses in R Studio (v4.3.2), following the pipeline proposed by Maksimovic et al [25], with modifications. This allowed us to perform more accurate data quality control, verifying the quality of extraction and the performance of the array technique. The processing involved normalizing raw data and quality control. Raw signal intensities were obtained from .idat files using the minfi Bioconductor R package (v1.48.0). Quality control involved the average detection p-value, flagging as low signal quality for averages >0.01. Filtering criteria included the removal of probes targeting the X and Y chromosomes, probes containing SNPs (Single-Nucleotide Polymorphisms), and cross-reactive probes.

Furthermore, a dissimilarity matrix plotted in a PCoA (Principal Coordinate Analysis) graph was generated post-processing to compare the methylation profiles of medulloblastomas and ependymomas. All graphs were created using the ggplot2 package (v3.4.4) in R Studio. Additionally, we submitted the entire case series to EpiDiP (Epigenomic Digital Pathology), an online platform developed by the Institute of Medical Genetics and Pathology, University Hospital Basel, and the University of Basel, Switzerland. EpiDiP was designed to assist in the diagnosis of brain tumors through methylation data analysis, complementing the Heidelberg Classifier. The platform considers only .idat files generated from Illumina Infinium Methylation 450K and EPIC arrays to plot CNV profiles and amplifications and fusions of genes relevant for rapid and accurate visualization. EpiDiP is available at <https://www.epidip.org>.

Considerations

In this study, DNA methylation profiling effectively confirmed and refined the diagnosis in 81% of cases initially evaluated by histopathology. This robust method provides a valuable diagnostic tool, particularly for challenging CNS tumors. Further refinement and validation of this approach could increase its clinical utility in the Brazilian public healthcare system, offering a more accurate and detailed understanding of CNS tumors and providing greater accuracy and depth in tumor classifications.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript." Please turn to the [CRediT taxonomy](#) for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section “MDPI Research Data Policies” at <https://www.mdpi.com/ethics>.

Acknowledgments: We would like to thank the Cytogenomics Laboratory team and the Division of Pathological Anatomy of HCFMUSP for their support and contributions to this study.

Conflicts of Interest: The authors of the manuscript entitled “Implementation of methylation profiling of central nervous system tumors at largest public health center in Brazil” declare that there are no conflicts of interest related to this work. All authors confirm that they have not received any funding or support from any institution or organization that could influence or have the potential to influence the content or interpretation of the results presented in this article. Furthermore, there are no financial, personal, or professional interests that could be interpreted as a potential conflict of interest.

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