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Impact of DNA Methylation Profiling on Central Nervous System Tumor Diagnosis and Management: A Pediatric Cohort Study from Türkiye

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ABSTRACT

AIM: To present a single-center experience, and to highlight the urgent need for multi-institutional collaboration in Türkiye and surrounding regions lacking access to methylation platforms, with the aim of enhancing diagnostic precision and neuropathological practice.

MATERIAL and METHODS: All pediatric patients who underwent methylation–based tumor classification (MBTC) between November 2023 and July 2025 were retrospectively identified. Clinical, histological, and molecular data were extracted and correlated with methylation results. Concordance between histopathology and MBTC was categorized as concordant, minor discordance, major discordance, novel classification, or un-classifiable.

RESULTS: A total of 48 tumors were profiled (26 females [54%]; 22 males [46%]; median age, 6.5 years; range, 0–17). The most frequent localization was supratentorial (n=18, 36%). Of the entire cohort, concordance was 58%. Excluding unclassifiable cases, concordance among evaluable tumors was 67%. Discordance occurred in 11 cases (23%), including 6 (13%) with major discrepancies. Concordance was significantly associated with tumor localization (p=0.028) but not WHO grade (p=0.17) and classifier confidence (p=0.73).

CONCLUSION: MBTC is a valuable complementary tool in the diagnostic workup of pediatric central nervous system (CNS) tumors, particularly in morphologically ambiguous and ultra-rare cases. It should be integrated with conventional histopathology rather than viewed as a replacement, as it may prevent prognostic misclassification and inappropriate treatment in selected patients.

KEYWORDS: DNA methylation, Pediatrics, Neuropathology, Glioma

ABBREVIATIONS: CNS: Central nervous system, **CNV:** Copy number variation, **FFPE:** Formalin-fixed paraffin-embedded, **IHC:** Immunohistochemistry, **ITD:** Internal tandem duplication, **MDB:** Medulloblastoma, **MBTC:** Methylation-based tumor classification, **NGS:** Next-generation sequencing, **PF:** Posterior fossa, **WHO:** World Health Organization

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INTRODUCTION

Accurate classification of pediatric central nervous system (CNS) tumors remains a major challenge due to their broad morphological heterogeneity and the growing number of newly recognized molecularly defined entities. DNA methylation profiling has emerged as an important complementary tool in this context.

Mapping and interpreting methylated regions with supervised machine learning classifiers has enabled methylation-based tumor classification (MBTC) of CNS tumors (5). Wiestler et al. conducted one of the earliest demonstrations, showing that methylation signatures could distinguish anaplastic gliomas independently of isocitrate dehydrogenase mutation or 1p/19q codeletion status (24). With continued development, MBTC has become a robust diagnostic adjunct applicable to formalin-fixed paraffin-embedded (FFPE) material, the global standard for pathological specimen preservation, and is now widely used to resolve diagnostic uncertainty in morphologically ambiguous cases (1).

Despite growing international adoption, MBTC implementation remains irregular, particularly in low- and middle-income countries where infrastructure, cost, and training barriers limit access to advanced molecular diagnostics. As a result, pediatric data from such regions are scarce, and the real-world impact of methylation profiling on diagnostic resolution is largely unknown outside high-income reference centers. Acibadem University is one of the few centers in Türkiye routinely applying methylation profiling to diagnostically challenging pediatric CNS tumors and receiving referrals from multiple provinces and external institutions.

Documenting this experience provides insight into the types of cases which most benefit from methylation-based analysis; the practical challenges encountered during implementation; and how this approach may reduce diagnostic variability at the national level. The aim of presenting this single-center experience is to contribute missing regional data to the international literature and support broader integration of molecular diagnostics into pediatric neuropathology practice.

MATERIAL and METHODS

All pediatric patients who underwent MBTC at our institution between November 2023 and July 2025 were retrospectively identified. MBTC was requested for tumors with indeterminate morphology or in cases of diagnostic disagreement between centers. As our institution functions as a national referral center, the cohort included both locally diagnosed cases and consultation cases from external hospitals.

DNA was extracted from FFPE tissue blocks at our institutional molecular pathology laboratory. All samples were processed locally up to the DNA extraction step. The extracted DNA was subsequently transferred to the distributor's accredited laboratory where bisulfite conversion, array hybridization, and scanning were performed using the Illumina Infinium MethylationEPIC BeadChip (850K) platform. Raw IDAT files generated by the external laboratory were electronically returned to our center. Quality control, normalization, and methylation-based classification were performed using the Epignostix/Heidelberg Brain Tumor Classifier version 12.8. The calibrated classifier score reflected model confidence; copy number variation (CNV) profiles were reviewed as complementary genomic data.

The following variables were recorded for each case: age, sex, tumor localization, histopathological diagnosis, assigned methylation class, World Health Organization (WHO) 2021 classification and grade, calibrated classifier score, and next-generation sequencing (NGS) results where available. Calibrated classifier scores were categorized based on established thresholds, consistent with the scoring framework introduced by Capper et al. for methylation-based CNS tumor classification (5): > 0.90 (high confidence), 0.50–0.90 (moderate confidence), and < 0.50 (low confidence).

Correlation between histopathology and MBTC was categorized as concordant, minor discordance, major discordance, novel class, or unclassifiable. As several discordance subcategories contained very small numbers, categories were dichotomized for statistical analysis. Cases were therefore grouped as either "concordant" or "non-concordant," with minor discordance, major discordance, and novel classes classified as non-concordant. Unclassifiable cases were excluded from concordance analysis.

Associations between concordance status and clinicopathological variables—including tumor localization (supratentorial, posterior fossa/cerebellum, ventricular, brainstem/spinal, or unknown), WHO grade (low grade [1–2] vs. high grade [3–4]), and classifier confidence—were evaluated using chi-square or Fisher's exact tests, as appropriate. Statistical analyses were performed in Python (SciPy, Pandas), with $p < 0.05$ considered statistically significant. Effect sizes were calculated using Cramér's V for χ^2 tests or the phi coefficient for 2 × 2 tables.

This retrospective study was approved by the Institutional Review Board of our institution (IRB No.: 2025-12/99). The requirement for informed consent was waived as no identifiable patient information was collected and all analyzed data were anonymized.

RESULTS

A total of 48 pediatric CNS tumors underwent MBTC between November 2023 and July 2025. The cohort encompassed the full pediatric spectrum (0–17 years of age) and reflected real-world referral patterns, including both primary and consultation cases (Table I). The cohort included 26 females (54%) and 22 males (46%), with a median age of 6.5 years. The most frequent tumor localization was supratentorial (cortical/subcortical) (n=18, 36%), followed by the posterior fossa/cerebellum (n=15, 31%), ventricular system (n=6, 13%), and brainstem/spinal cord (n=5, 10%); localization was undocumented or uncertain in 4 cases (8%). The tumor localization distribution is illustrated in Figure 1. NGS data were available for 26 tumors (54%), primarily to evaluate clinically relevant and potentially targetable alterations.

Table I: Summary of Study Cohort

Category	Methylation Group	Methylation Subtype	Number of Cases	Median Age (years)	Localizations	WHO Grade
Adult-type diffuse gliomas	Glioblastoma, IDH-wild type	Mesenchymal subtype	1	15	Frontal lobe	4
Pediatric-type diffuse low-grade gliomas	Angiocentric glioma, MYB/MBL1 altered		1	8	Temporal lobe	1
	Diffuse glioma, MAPK altered, cell cycle activated	Diffuse glioma, MAPK altered, cell cycle activated	1	4	Temporal lobe	1
Pediatric-type diffuse high-grade gliomas	Diffuse midline glioma, H3 K27-altered, subtype H3 K27-mutant or EZHIP expressing	Diffuse midline glioma, H3 K27-altered, subtype H3 K27-mutant or EZHIP expressing	3	9	Cervical spine, Pons	4
	Diffuse pediatric-type high grade glioma, MYCN subtype	Diffuse pediatric-type high grade glioma, MYCN subtype	2	11.5	Frontotemporal lobe, Temporal lobe	4
	Diffuse pediatric-type high grade glioma, RTK1 subtype, subclass A	Diffuse pediatric-type high grade glioma, RTK1 subtype, subclass A	1	8	Frontal lobe	4
Circumscribed astrocytic gliomas	Pilocytic astrocytomal	Hemispheric	1	9	Frontal lobe	1
		Midline	2	6	Third ventricle, Globus pallidus	1
		Infratentorial	2	10.5	Cerebellum	1
Glioneuronal and neuronal tumors	Ganglioglioma		1	3	Temporal lobe	1
	Dysembryoplastic neuroepithelial tumor		1	3	Temporal lobe	1
	Papillary glioneuronal tumor; PRKCA-fused		1	17	Lateral ventricle	1
Ependymal tumors	Posterior fossa group B ependymoma	Subclass 2	1	12	Posterior fossa	3
	Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused	Subclass A	2	6.5	Parietal lobe, unknown	3
Choroid plexus	Choroid plexus papilloma	Pediatric subtype	1	0	Posterior fossa	2
Embryonal	Medulloblastoma	Group 3 subtype	1	1	Cerebellum	4
		Group 3 subtype, subclass I	1	7		4
		Group 3 subtype, subclass IV	1	4		4
		Group 4 Subtype, Subclass VI	1	8		4
		Group 4 subtype, subclass VII	4	8		4
		Group 4 subtype, subclass VIII	1	4		4
	Atypical teratoid/ rhabdoid tumor	MYC-subtype	1	9	Posterior fossa	4
		TYR subtype	2	2	Posterior fossa, Lateral ventricle	4
	CNS tumor with BCOR internal tandem duplication		1	7	Left hemisphere	Unclassified
	Embryonal tumor with multilayered rosettes, C19MC-altered		3	3	Parietal lobe, Pons	4
Mesenchymal, non-meningothelial tumors involving the CNS	CIC-rearranged sarcoma		3	4	Parietal lobe	4
Novel	Neuroepithelial tumor with PATZ1 fusion		1	8	Lateral ventricle	Unclassified
	Neuroepithelial tumor, MN1:CXXC5-fused		1	14	Intraventricular	
	Neuroepithelial tumor, PLAGL1-fused		1	6	Temporal lobe	
Benign	Unclassified (Masson Tumor)		1	0	Supratentorial	Unclassified
Other	Unclassified/Unclassifiable		4	8	Frontal lobe, Posterior fossa	Inadequate DNA

WHO 2021 grading was applicable in most cases: 25 tumors (51%) were Grade 4; 3 (6%) were Grade 3; 1 (2%) was Grade 2; and 10 (21%) were Grade 1. In total, 4 tumors (8%) were unclassified according to WHO 2021, with 4 (8%) unclassifiable due to low tissue quality. One tumor (2%) was a benign vascular lesion not represented in the WHO CNS classification.

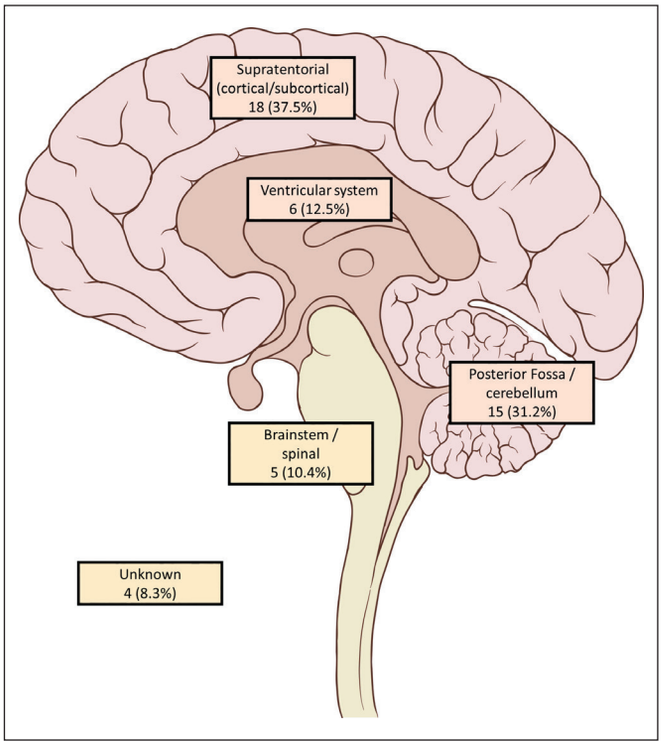

Figure 1: Tumor Localizations. The most frequent tumor localization was supratentorial (cortical/subcortical) (n=18, 36%), followed by the posterior fossa/cerebellum (n=15, 31%), ventricular system (n=6, 13%), and brainstem/spinal (n=5, 10%); localization was undocumented or uncertain in 4 cases (8%).

The classifier yielded a mean calibrated score of 0.84 (range, 0.30–1.00; median, 0.98). Classification confidence was high in 27 cases (56%), moderate in 11 cases (23%), and low in 6 cases (13%). All 4 unclassifiable tumors had calibrated scores below 0.30.

In terms of final classification, 40 tumors (83%) corresponded to WHO-recognized entities, 3 tumors (6%) represented novel methylation classes, and 5 tumors (10%)—4 malignant and 1 benign—were unclassifiable. Of the WHO-recognized tumors, 3 of 40 cases (7.5%; equivalent to 6% of the entire cohort) showed discordance between histopathological and methylation-based diagnosis, and 8 tumors (17%) were assigned to methylation subtypes not currently included in the WHO 2021 classification.

Correlation between histopathology and MBTC was concordant in 28 tumors (58%). Minor discordance was observed in 5 cases (10%), major discordance in 6 cases (13%), and 3 tumors (6%) were assigned to novel methylation classes, all of which were necessarily non-concordant. Concordance could not be assessed in 6 cases (13%) due to insufficient material or unclassifiable methylation profiles. For dichotomized analysis, cases with unclassifiable profiles (n = 6) were excluded. Of the remaining 42 evaluable tumors, 28 (67%) were concordant and 14 (33%) were non-concordant.

Concordance was not significantly associated with WHO grade (low grade [1–2] vs. high grade [3–4]; $\chi^2 = 1.86$, $p = 0.17$; $\phi = 0.21$, small effect). However, concordance was significantly associated with tumor localization ($\chi^2 = 9.08$, df = 3, $p = 0.028$). Posterior fossa tumors showed the highest concordance (15/16, 94%). Supratentorial (9/17, 53%) and ventricular tumors (2/5, 40%) were more frequently discordant. The effect size was in the medium-to-large range (Cramér's V = 0.48). Concordance was not significantly associated with classifier confidence ($\chi^2 = 0.88$, df = 2, $p = 0.65$), and the effect size was small (Cramér's V = 0.12). Concordance distributions are illustrated in Figure 2.

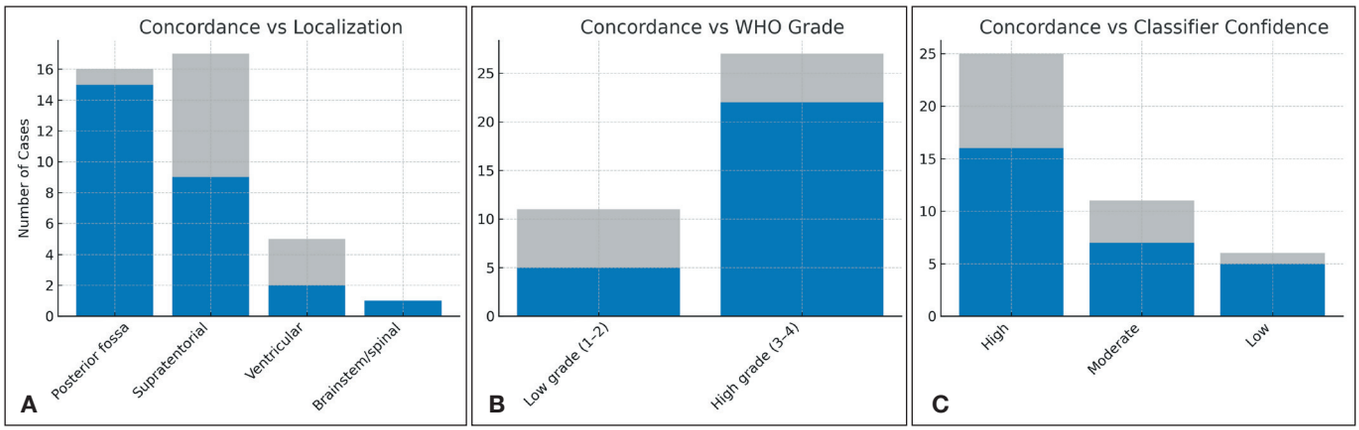

Figure 2: Concordance between histopathology and methylation-based tumor classification (MBTC) according to tumor localization **(A)**, WHO grade **(B)**, and classifier confidence **(C)**. Localization showed a significant association with concordance (p = 0.028), whereas neither WHO grade (p = 0.17) nor classifier confidence (p = 0.65) demonstrated significant associations. Blue bars indicate concordant cases and gray bars indicate non-concordant cases.

Concordance with histopathology was highest in medulloblastomas (9/10, 90%). Discordance was more common in diffuse gliomas (5/7 non-concordant, 71%) and ependymomas (1/3 non-concordant, 33%), reflecting their greater morphological and molecular heterogeneity. All 3 novel methylation-defined tumors (100%) were discordant by definition. In total, 6 tumors (13%) could not be classified by MBTC due to low-quality or atypical DNA methylation profiles.

DISCUSSION

This study is the first reported clinical experience with MBTC of CNS tumors in Türkiye. At the time of the study, only a single center in the country had established the capacity to perform this analysis. MBTC has both advantages and limitations, as with all diagnostic modalities. Its chief strength lies in providing diagnostic clarity in otherwise ambiguous cases, informing appropriate clinical management (5). While accurate classification is essential for determining therapy and prognosis, a limitation of MBTC is that it does not by itself identify actionable variants. In over half of the study cohort, MBTC was complemented by concurrent NGS which provided additional information on clinically relevant and potentially targetable alterations. This illustrates the value of integrated molecular diagnostics in pediatric neuro-oncology. Nevertheless, by refining the understanding of tumor pathogenesis, methylation profiling may facilitate the discovery of novel therapeutic avenues more efficiently and cost-effectively.

Diagnostic challenges are pronounced in pediatric CNS tumors due to their marked morphological heterogeneity. Histologically similar appearances may represent biologically distinct entities—for example, gangliogliomas with pilocytic features—or, conversely, a single entity such as pilocytic astrocytoma may show diverse morphologies including oligodendrocyte-like cells. Pattern recognition supported by a targeted immunohistochemical (IHC) panel is sufficient for most tumors in routine practice, as demonstrated in recent regional studies (10,13,18). However, in cases where morphology is equivocal and limited IHC does not yield clarity, MBTC offers an adjunctive solution especially valuable in the pediatric setting where accurate classification has direct therapeutic and prognostic implications.

From a practical standpoint, the input requirements for methylation profiling are broadly comparable to those of large DNA + RNA NGS panels. Both approaches generally require a DNA input of approximately 200–500 ng from FFPE or frozen material (9). A key distinction is that methylation profiling does not require RNA, which is often difficult to obtain in sufficient quality from small biopsies or archival samples. This increases feasibility in pediatric neuro-oncology, where tissue quantity may be limited. Turnaround time is comparable to that of large NGS panels and depends on batching and bioinformatics capacity. Cost per sample is typically lower due to the absence of RNA extraction and library preparation. These practical features may reduce technical barriers in laboratories developing molecular workflows and increase the likelihood of successful implementation, particularly in centers handling small or fragmented diagnostic specimens.

To provide a coherent framework, entity-specific findings were grouped into three domains: (i) established pediatric tumor types where MBTC directly informs clinical risk stratification (e.g., medulloblastoma [MDB]), (ii) emerging methylation classes not incorporated into the current WHO classification, and (iii) morphologically ambiguous tumors for which MBTC offers diagnostic resolution.

MDB represents one of the most important pediatric groups in which MBTC has immediate clinical utility. While WNT- and SHH-activated tumors can be identified using IHC, non-WNT/non-SHH tumors cannot be reliably subclassified without molecular profiling (2,7). Approximately 60% of all MDBs fall within the so-called Group 3/4 category, further subdivided into at least 8 methylation-defined subgroups which cannot be reliably distinguished by histology or conventional techniques alone. These molecularly defined subgroups display distinct biological behavior, including varying metastatic potential and characteristic driver events, with *MYC* or *MYCN* amplification emerging as key CNVs associated with aggressive clinical course (16,21). One limitation of MBTC, however, is that it does not provide information on *TP53* mutational status, which remains clinically relevant in SHH-activated (Group 2) MDB. In this pediatric cohort, MDB accounted for 10 of 48 tumors (21%). In 9 of these cases (90%), MBTC provided critical diagnostic refinement not achievable through histology and IHC alone. The only unclassifiable case was a consultation block, likely affected by pre-analytic tissue quality. This finding underscores the practical value of methylation profiling in the routine evaluation of pediatric MDB.

Beyond the established WHO 2021 entities, MBTC occasionally identifies tumor classes not yet formally recognized. In this pediatric cohort, 3 tumors (6.2%) were assigned to novel methylation classes: neuroepithelial tumor with *PATZ1* fusion; neuroepithelial tumor with *MN1::CXXC5* fusion; and neuroepithelial tumor with *PLAGL1* fusion. The case classified as a *PATZ1*-fused tumor showed histological features resembling a supratentorial ependymoma, Grade 3. NGS confirmed a *MN1::PATZ1* fusion. The *MN1::CXXC5*-fused tumor was initially interpreted as a high-grade neuroepithelial tumor at the referring laboratory. Despite that both harbored *MN1* fusions, their histological appearances were markedly diverse (Figure 3A and 3B). The *PLAGL1*-fused tumor was described as a low-grade glioneuronal tumor in its original pathology report. The morphology is illustrated in Figure 3C. These examples highlight how methylation profiling can reveal underlying biological distinctions not apparent by morphology alone, emphasizing the evolving nature of CNS tumor taxonomy and underscoring the role of MBTC in expanding the diagnostic framework (1,8,15,22). Nevertheless, interpretation of these novel classes requires caution, as their long-term clinical behavior and optimal management strategies remain to be fully defined.

MBTC also frequently assigns tumors to subclasses which exceed the granularity of WHO 2021. In this cohort, pilocytic astrocytomas were subclassified into infratentorial/posterior fossa, midline, and hemispheric groups. Similarly, ependymomas were resolved into supratentorial *ZFTA* fusion-positive subclasses (including ZFTA–RELA subclass A) and posterior

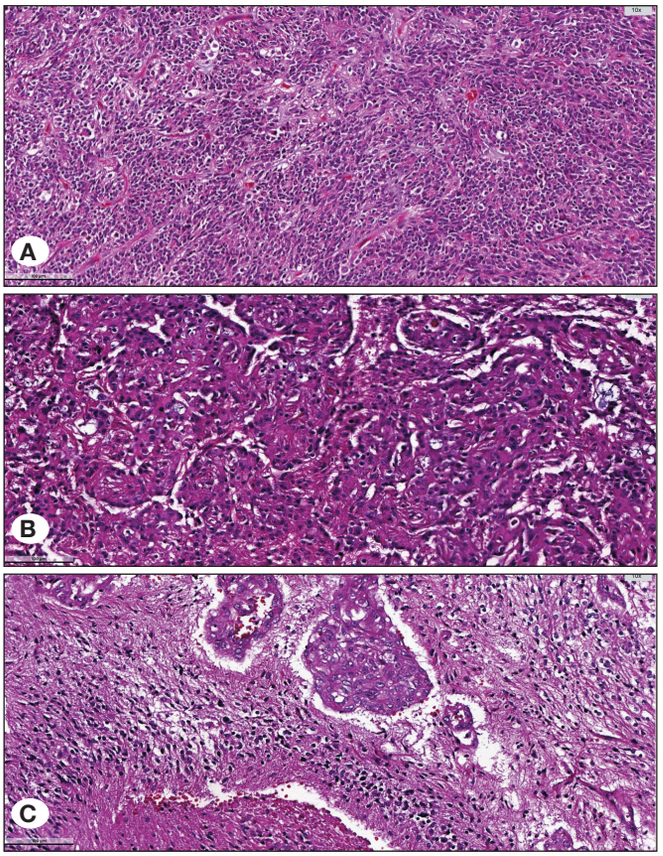

Figure 3: Histological micrographs of novel tumors not classified in the 2021 WHO classification of CNS tumors. **A)** Neuroepithelial tumor with PATZ1 fusion. **B)** Neuroepithelial tumor with MN1::CXXC5 fusion. **C)** Neuroepithelial tumor with PLAGL1 fusion. Hematoxylin and eosin, magnification ×100.

fossa group B (PFB-2). While WHO 2021 distinguishes major molecular groups such as ST-E *ZFTA* fusion-positive and PFA vs. PFB, it does not currently endorse these deeper subclass layers (3). Such subclassifications, available in the DKFZ/Heidelberg classifier (v12.8), may support future risk stratification and trial design. *ZFTA*-fusion–positive tumors are shown to comprise several molecular clusters, including those involving *NCOA1/2* or *MAML2* partners, although these remain investigational (17, 23). Cavalli et al. identified 5 PFB subclasses, with poorer survival restricted to PFB1 and PFB3 (6). Subclass distinctions did not alter initial management in this cohort but may hold future prognostic significance.

A compelling example of the diagnostic utility of MBTC arises in ultra-rare entities. Tumors previously grouped under primitive neuroectodermal tumors are now recognized as distinct molecularly defined embryonal neoplasms, including those driven by *CIC*, *FOXR2*, *MN1*, and *BCOR* alterations (19). Such alterations may be missed by small targeted NGS panels; broader sequencing approaches are often unavailable or cost prohibitive. In this cohort, one 7-year-old girl with a hemispheric mass—previously diagnosed as a high-grade ependymoma—was reclassified by MBTC as a CNS tumor with BCOR internal tandem duplication (ITD). Notably, CNS BCOR-ITD tumors are so newly defined that no formal WHO grade assignment currently exists. Similarly, three 4-year-old girls originally diagnosed with high-grade glioma were reassigned by MBTC to CIC-rearranged sarcoma with high confidence. These major discordant cases shared two unifying features: morphology which overlapped with common pediatric glial tumors, leading to plausible but incorrect histological diagnoses; and IHC profiles insufficiently specific to distinguish between biologically unrelated entities. For example, BCOR-ITD may be undetectable on IHC as BCOR staining can be negative despite the underlying alteration (20). These cases highlight a recurring pitfall in pediatric neuro-oncology: certain ultra-rare molecular entities can convincingly mimic high-grade gliomas or embryonal tumors on routine workup. MBTC therefore serves as a critical safeguard against prognostically significant misclassifications.

Importantly, several reclassified tumors would have received substantially different treatments had MBTC not been performed. High-grade ependymoma is typically treated with adjuvant radiotherapy. CNS BCOR-ITD tumors are biologically distinct and generally managed with intensive chemotherapy-based regimens (14). Thus, without MBTC, the 7-year-old girl with BCOR-ITD would likely have undergone inappropriate radiotherapy-based management. Likewise, the two tumors originally labeled as high-grade glioma but reclassified as CIC-rearranged sarcoma would have been placed on glioma protocols. CIC-rearranged sarcomas, however, require sarcoma-directed treatment strategies, not glioma regimens (4).

Glioneuronal tumors represent another area of diagnostic difficulty in both histopathology and MBTC. In this cohort, two such tumors were assigned to low-confidence or alternative classes. A previous work reported an 11-year-old girl whose lesion was interpreted as a high-grade glioneuronal tumor but was reclassified by MBTC as pilocytic astrocytoma, midline, with a high confidence score (25). This discrepancy has major clinical implications, as a diagnosis of high-grade glioma would prompt aggressive therapy, whereas pilocytic astrocytoma has a markedly better prognosis. Similar challenges have been documented in larger institutional series, emphasizing that glioneuronal tumors frequently cluster ambiguously or fail to reach a confident methylation assignment (12). These findings highlight the need for an integrated diagnostic approach combining morphology, IHC, molecular alterations, and clinical context.

A practical limitation of MBTC is sensitivity to tissue quality. In this series, low-quality consultation material—often representing small blocks with suboptimal fixation—produced failed or unclassifiable profiles. This underscores the importance of proper fixation, processing, and DNA preservation, particularly where rare pediatric tumors are referred for tertiary evaluation. Importantly, this limitation is not specific to MBTC but applies to all molecular assays. Recognizing these pre-analytic constraints is crucial for realistic integration of MBTC into routine practice.

Beyond technical considerations, broader implementation of MBTC in Türkiye presents financial and systemic obstacles. At present, MBTC is not reimbursed by the national social security system, and the cost—approximately equal to the national minimum monthly wage—must be paid by patients, creating substantial barriers to access. Addressing reimbursement, infrastructure, and inter-institutional collaboration is essential for integrating MBTC into routine pediatric neuro-oncology care. This work highlights the feasibility and diagnostic value of MBTC and may help lay the groundwork for national adoption.

A strength of this study is that our center functions as a national reference hub, receiving consultation material and referral cases from across Türkiye and neighbor countries. This contributed to the diversity of the cohort, encompassing a wide spectrum of pediatric CNS tumors rather than a narrowly selected institutional population. Although this cohort included 48 cases over less than 2 years, this number is consistent with other institutional experiences and is meaningful in the context of rare pediatric CNS tumors. Indeed, prior reports, including Karimi et al., have demonstrated that even moderate case volumes can yield impactful insights, provided that they originate from referral-based series (11). Such diversity increases the representativeness of these findings; however, broader multi-institutional studies remain necessary to validate and expand these results.

CONCLUSION

This experience demonstrates that MBTC is a valuable adjunct in the diagnostic workup of pediatric CNS tumors, particularly in morphologically ambiguous and ultra-rare entities. However, its current limitations include uncertain performance in certain glioneuronal tumors, subclassifications which are not currently clinically actionable, and susceptibility to pre-analytic factors. MBTC should therefore be regarded neither as a replacement for conventional histopathology nor a universal solution, but as a complementary tool within an integrated diagnostic framework. Applied in this balanced manner, MBTC could potentially enhance diagnostic precision while minimizing the risk of over-reliance or over-interpretation. As classification systems evolve and molecular approaches converge, MBTC is likely to become an increasingly indispensable component of precision neuropathology.

Declarations

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Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author by reasonable request.

Disclosure: The authors declare no competing interests.

AUTHORSHIP CONTRIBUTION

Study conception and design: MAI, AED

Data collection: BT, BB, FS, MO

Analysis and interpretation of results: MAI, AED

Draft manuscript preparation: MAI

Critical revision of the article: AED, BT, MO

Other (study supervision, fundings, materials, etc...): BB, FS, BT, MO

All authors (MAI, AED, BT, BB, FS, MO) reviewed the results and approved the final version of the manuscript.

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Supplementary Table: Clinicopathological and Molecular Characteristics of the Study Cohort.

Patient Number	Localization	Histological Diagnosis	Methylation Profile	Correlation with Histology	WHO Class	Score	WHO Class	NGS Result	Age	Gender	WHO Grade
1	Right temporal lobe	Consultation (Angiocentric glioma)	Angiocentric glioma, MYB/MBL1 altered	Concordant	Angiocentric glioma	0.99	Existing	MYB::QKI	8	Female	1
2	Posterior fossa	Consultation (Unknown diagnosis)	Atypical teratoid/ rhabdoid tumour, MYC-subtype	Unknown	Atypical teratoid/ rhabdoid tumour, MYC-subtype	0.99	Existing	Not tested	9	Male	4
3	Posterior fossa	Atypical teratoid/ rhabdoid tumour	Atypical teratoid/ rhabdoid tumour, TYR subtype	Concordant	Atypical teratoid/ rhabdoid tumour, TYR-subtype	0.98	Existing	SMARCB1	0	Female	4
4	Right lateral ventricule	Atypical teratoid/ rhabdoid tumour	Atypical teratoid/ rhabdoid tumour, TYR subtype	Concordant	Atypical teratoid/ rhabdoid tumour, TYR subtype	0.81	Existing	Not tested	4	Female	4
5	Posterior fossa	Atypical choroid plexus papilloma	Choroid plexus papilloma, pediatric subtype	Non-concordant (Minor)	Atypical choroid plexus papilloma	0.9	Existing (Discordant)	Not tested	0	Female	2
6	Left parietal lobe	High grade pediatric glioma	CIC-rearranged sarcoma	Non-concordant (Major)	CIC-rearranged sarcoma	0.99	Existing	Negative (Not in panel)	4	Female	4
7	Left parietal lobe	High grade glial tumor	CIC-rearranged sarcoma	Non-concordant (Major)	CIC-rearranged sarcoma	0.99	Existing	Not tested	4	Female	4
8	Left parietal lobe	High grade glial tumor	CIC-rearranged sarcoma	Non-concordant (Major)	CIC-rearranged sarcoma	0.99	Existing	Not tested	4	Female	4
9	Left hemisphere	High grade neuroepithelial tumour	CNS tumour with BCOR internal tandem duplication	Non-concordant (Major)	CNS tumour with BCOR internal tandem duplication	0.99	Existing	Not tested	7	Female	Unclassified
10	Temporal lobe	High grade glioneuronal tumor	Diffuse glioma, MAPK altered, cell cycle activated	Non-concordant (Minor)	Ganglioglioma with anaplastic features	0.31	Existing (Discordant)	CDKN2A loss	4	Male	1
11	Pons	Diffuse midline glioma, H3.3 K27–mutant	Diffuse midline glioma, H3 K27-altered, subtype H3 K27-mutant or EZHIP expressing	Concordant	Diffuse midline glioma, H3.3 K27–mutant	0.89	Existing	H3K27M, IDH2 and TP53	6	Female	4
12	Talamus	High grade glioma, H3.3 K27–mutant	Diffuse midline glioma, H3 K27-altered, subtype H3 K27-mutant or EZHIP expressing	Concordant	Diffuse midline glioma, H3.3 K27–mutant	0.99	Existing	H3K27M and PIK3CA	9	Female	4

Supplementary Table: Cont.

Patient Number	Localization	Histological Diagnosis	Methylation Profile	Correlation with Histology	WHO Class	Score	WHO Class	NGS Result	Age	Gender	WHO Grade
13	Cervical spine	Diffuse midline glioma, H3.3 K27–mutant	Diffuse midline glioma, H3 K27-altered, subtype H3 K27-mutant or EZHIP expressing	Concordant	Diffuse midline glioma, H3.3 K27–mutant	0.99	Existing	H3K27M and TP53; CDKN2A/B and RB1 deletion	12	Male	4
14	Temporal lobe	Consultation (High grade neuroepithelial tumor)	Diffuse paediatric-type high grade glioma, MYCN subtype	Concordant	Diffuse paediatric-type high-grade glioma MYCN	0.9	Existing	PTEN deletion, N-MYC amplification	9	Female	4
15	Right frontotemporal lobe	Consultation (High grade glial tumour)	Diffuse paediatric-type high grade glioma, MYCN subtype	Concordant	Diffuse paediatric-type high-grade glioma MYCN	0.4	Existing	Negative	14	Male	4
16	Left frontal lobe	High grade glial tumor	Diffuse paediatric-type high grade glioma, RTK1 subtype, subclass A (novel)	Concordant	Diffuse paediatric-type high-grade glioma RTK1	0.48	Existing	dMMR	8	Male	4
17	Temporal lobe	Low grade mixt glioneuronal tumor (DNET+RFGNT)	Dysembryoplastic neuroepithelial tumour	Concordant	Dysembryoplastic neuroepithelial tumour	0.98	Existing	FGFR1::TACC1	3	Male	1
18	Right frontoparietal lobe	Consultation (Unknown diagnosis)	Embryonal tumour with multilayered rosettes, C19MC-altered	Unknown	Embryonal tumour with multilayered rosettes, C19MC-altered	1	Existing	Not tested	2	Male	4
19	Pons	Consultation (Embryonel tumour)	Embryonal tumour with multilayered rosettes, C19MC-altered	Concordant	Embryonal tumour with multilayered rosettes, C19MC-altered	0.98	Existing	Not tested	3	Male	4
20	Right frontoparietal lobe	Consultation (Embryonel tumor)	Embryonal tumour with multilayered rosettes, C19MC-altered	Concordant	Embryonal tumour with multilayered rosettes, C19MC-altered	1	Existing	Not tested	3	Male	4
21	Temporal lobe	Consultation (Ped. Low Grade Glioma)	Ganglioglioma	Non-concordant (Minor)	Ganglioglioma	0.99	Existing	BRAF V600E	3	Female	1
22	Left frontal lobe	High grade glial tumor	Glioblastoma, IDH-wildtype, mesenchymal subtype	Concordant	Glioblastoma, IDH-wildtype	0.55	Existing (Unclassified subtype)	RB1, TP53	15	Female	4
23	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 3 subtype	Concordant	Medulloblastoma, Group 3 subtype	0.46	Existing	Not tested	1	Male	4
24	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 3 subtype, subclass I	Concordant	Medulloblastoma, Group 3 subtype	0.94	Existing	Not tested	7	Male	4

Supplementary Table: Cont.

Patient Number	Localization	Histological Diagnosis	Methylation Profile	Correlation with Histology	WHO Class	Score	WHO Class	NGS Result	Age	Gender	WHO Grade
25	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 3 subtype, subclass IV	Concordant	Medulloblastoma, Group 3 subtype	0.99	Existing	Negative	4	Male	4
26	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 4 Subtype, Subclass VI	Concordant	Medulloblastoma, Group 4 subtype	0.98	Existing	Not tested	8	Male	4
27	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 4 subtype, subclass VII	Concordant	Medulloblastoma, Group 4 subtype	0.86	Existing	Negative	5	Female	4
28	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 4 subtype, subclass VII	Concordant	Medulloblastoma, Group 4 subtype	0.99	Existing	Not tested	7	Male	4
29	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 4 subtype, subclass VII	Concordant	Medulloblastoma, Group 4 subtype	0.99	Existing	Not tested	9	Female	4
30	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 4 subtype, subclass VII	Concordant	Medulloblastoma, Group 4 subtype	0.99	Existing	Not tested	9	Female	4
31	Posterior fossa	Classical Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, Group 4 subtype, subclass VIII	Concordant	Medulloblastoma, Group 4 subtype	0.94	Existing	Not tested	4	Male	4
32	Left lateral ventricule	Supratentorial ependymoma, grade 3	Neuroepithelial tumour with PATZ1 fusion (novel)	Non-concordant (Novel)	Unclassified	0.9	Novel	MN1::PATZ1	8	Male	Unclassified
33	Intraventriculer	Consultation (High grade neuroepithelial tumour)	Neuroepithelial tumour, MN1:CXXC5-fused (novel)	Non-concordant (Novel)	Astroblastoma, MN1-altered	0.78	Novel	MN1::CXXC5	14	Male	Unclassified
34	Temporal lobe	Consultation (Low grade glioneuronal tumor)	Neuroepithelial tumour, PLAGL1-fused (novel)	Non-concordant (Novel)	Supratentorial ependymoma, NOS	0.52	Novel	Not tested	6	Female	Unclassified
35	Right lateral ventricule	Papillary glioneuronal tumour	Papillary glioneuronal tumour; PRKCA-fused	Concordant	Papillary glioneuronal tumour	0.74	Existing	Negative	17	Female	1
36	Frontal lobe	Glioneuronal tumour	Pilocytic astrocytoma, hemsipheric	Non-concordant (Major)	Pilocytic astrocytoma with features of anaplasia	0.99	Existing (Discordant)	BRAF::KIAA1549, BRAF::HIP1	9	Female	1

Supplementary Table: Cont.

Patient Number	Localization	Histological Diagnosis	Methylation Profile	Correlation with Histology	WHO Class	Score	WHO Class	NGS Result	Age	Gender	WHO Grade
37	Cerebellum	Pilocytic astrocytoma	Pilocytic astrocytoma, infratentorial	Concordant	Pilocytic astrocytoma	0.99	Existing (Unclassified subtype)	BRAF::KIAA1549	5	Female	1
38	Posterior fossa	Pilocytic astrocytoma	Pilocytic astrocytoma, infratentorial	Concordant	Pilocytic astrocytoma	0.64	Existing (Unclassified subtype)	BRAF::KIAA1549	16	Male	1
39	Third ventricle	Pilomyxoid astrocytoma	Pilocytic astrocytoma, midline	Non-concordant (Minor)	Pilocytic astrocytoma	0.99	Existing (Unclassified subtype)	BRAF::KIAA1549	1	Female	1
40	Left globus pallidus	High grade glial tumor	Pilocytic astrocytoma, midline	Non-concordant (Major)	Pilocytic astrocytoma	0.98	Existing (Unclassified subtype)	NF1	11	Female	1
41	Posterior fossa	Consultation (Posterior fossa ependymoma, grade 3)	Posterior Fossa Group B (pfb) Ependymoma, Subclass 2 (novel)	Concordant	Posterior Fossa Ependymoma, Group B	0.99	Existing (Unclassified subtype)	Not tested	12	Male	3
42	Right frontoparietal lobe	Supratentorial ependymoma, grade 3	Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused, subclass A (novel)	Concordant	Supratentorial ependymoma, ZFTA fusion–positive	0.3	Existing (Unclassified subtype)	CDKN2A/2B loss, MDM4 amplification	5	Female	3
43	Unknown	Consultation (Supratentorial ependymoma, grade 3)	Supratentorial ependymoma, ZFTA fusion-positive, subtype ZFTA-RELA fused, subclass A (novel)	Non-concordant (Minor)	Supratentorial ependymoma, ZFTA fusion–positive	0.99	Existing (Unclassified subtype)	Not tested	8	Male	3
44	Lateral ventricle	High grade neuroepithelial tumour	Unclassifiable	Unknown	Unclassifiable		Unclassifiable	Not tested	2	Male	Unclassifiable
45	Supratentoriyal	Masson Tumour	Unclassified	Concordant	Benign		Unclassifiable (Benign)	Negative	0	Female	Benign
46	Brain stem	Paediatric-type diffuse low-grade glioma	Unclassified (Low tumor content)	Unknown	Unclassifiable		Unclassifiable	Negative	8	Female	Unclassifiable
47	Cervical spine	Diffuse glial tumour	Unclassified (Low tumor content)	Unknown	Unclassifiable		Unclassifiable	Not tested	17	Female	Unclassifiable
48	Unknown	Consultation (Medulloblastoma)	Unclassified (Poor tissue quality)	Unknown	Unclassifiable	0.3	Unclassifiable	Not tested	2	Male	Unclassifiable