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Case Report

Utility of Comprehensive Genomic Profiling for Precise Diagnosis of Pediatric-Type Diffuse High-Grade Glioma

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In the current World Health Organization classification of central nervous system tumors, comprehensive genetic and epigenetic analyses are considered essential for precise diagnosis. A 14-year-old male patient who presented with a cerebellar tumor was initially diagnosed with glioblastoma and treated with radiation and concomitant temozolomide chemotherapy after resection. During maintenance temozolomide therapy, a new contrast-enhanced lesion developed in the bottom of the cavity formed by the resection. A second surgery was performed, but the histological findings in specimens from the second surgery were different from those of the first surgery. Although genome-wide DNA methylation profiling was conducted using frozen tissue for a precise diagnosis, the proportion of tumor cells was insufficient and only normal cerebellum was observed. We then performed comprehensive genetic analysis using formalin-fixed paraffin-embedded sections, which revealed *MYCN* amplification without alteration of *IDH1*, *IDH2*, or *Histone H3*. Finally, the patient was diagnosed with pediatric-type diffuse high-grade glioma, H3-wildtype and IDH-wildtype. In conclusion, comprehensive genetic and epigenetic analysis should be considered in pediatric brain tumor cases.

Key words: comprehensive genomic profiling, pediatric brain tumor, genome-wide DNA methylation, MYCN

Neoplasms of the central nervous system are the second most common malignancy and the leading cause of cancer-related death in childhood [1]. The current World Health Organization (WHO) classification for the central nervous system (CNS) defines pediatric-type diffuse gliomas as high-grade or low-grade based on morphological features and genetic and epigenetic alterations. High-grade gliomas include diffuse midline glioma; H3 K27-altered, diffuse hemispheric glioma; H3 G34-mutant, pediatric-type diffuse high-grade glioma; H3-wildtype and IDH-wildtype gliomas; and infant-type hemispheric glioma. Accurate diagnosis leads to more precise treatment and a better prognosis, but diagnosing pediatric brain tumors is challenging.

The recent development of novel technologies including comprehensive genomic and epigenomic profiling have greatly improved CNS tumor diagnostics. DNA methylation profiling, which assesses the methylation status of CpG sites across the entire human genome, is the most powerful diagnostic tool yet developed, and the German Cancer Research Center (DKFZ) and Heidelberg University [2] offer an online public database of CNS tumor methylation profiles. More than 50% of the diagnostic entries in the current WHO classification

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include DNA methylation profiles as an essential or recommended diagnostic feature [3]. Indeed, our team and other researchers have shown the utility of DNA methylation profiling in pediatric brain tumors [4,5]. FoundationOne® CDx (F1CDx) is another comprehensive genomic profiling tool and a next-generation sequencing-based assay that was recently approved by the Ministry of Health, Labour and Welfare (MHLW) in Japan as a comprehensive genomic profiling test for solid tumors, and its utility for CNS tumors has been reported [6,7]. F1CDx has been shown to promote diagnostic accuracy and enhance clinical decision-making in pediatric low- and high-grade gliomas [8].

In this report, we present the case of a 14-year-old male patient who was initially diagnosed with glioblastoma not otherwise specified (NOS) and treated with adjuvant chemoradiotherapy including temozolomide after surgery. In a recurrent lesion 13 months later, the pathological findings were incompatible with the initial lesion; therefore, comprehensive genetic analysis was conducted. F1CDx revealed amplification of *MYCN*, which led to the final diagnosis of pediatric-type diffuse high-grade glioma, H3-wildtype and IDH-wildtype, in accordance with the current WHO classification.

Case Report

The patient was a 14-year-old male presenting with headache and vomiting for several days. His head magnetic resonance imaging (MRI) revealed a ring-enhanced irregular tumor in the right cerebellar hemisphere with surrounding fluid-attenuated inversion recovery (FLAIR) hyperintense signal (Fig. 1A-E). The fourth ventricle was compressed by the tumor, and the patient also had obstructive hydrocephalus. External ventricular drainage and tumor resection were performed. The patient underwent occipital craniotomy. The tumor was covered by the normal cerebellar cortex, and a corticotomy was performed. The border between the tumor and normal parenchyma was not well-delineated, and the tumor was soft and hypervascular in nature (Fig. 1F). The patient could not ingest oral 5-aminolevulinic acid (5-ALA) due to nausea, so intraoperative 5-ALA fluorescence-guided resection was not

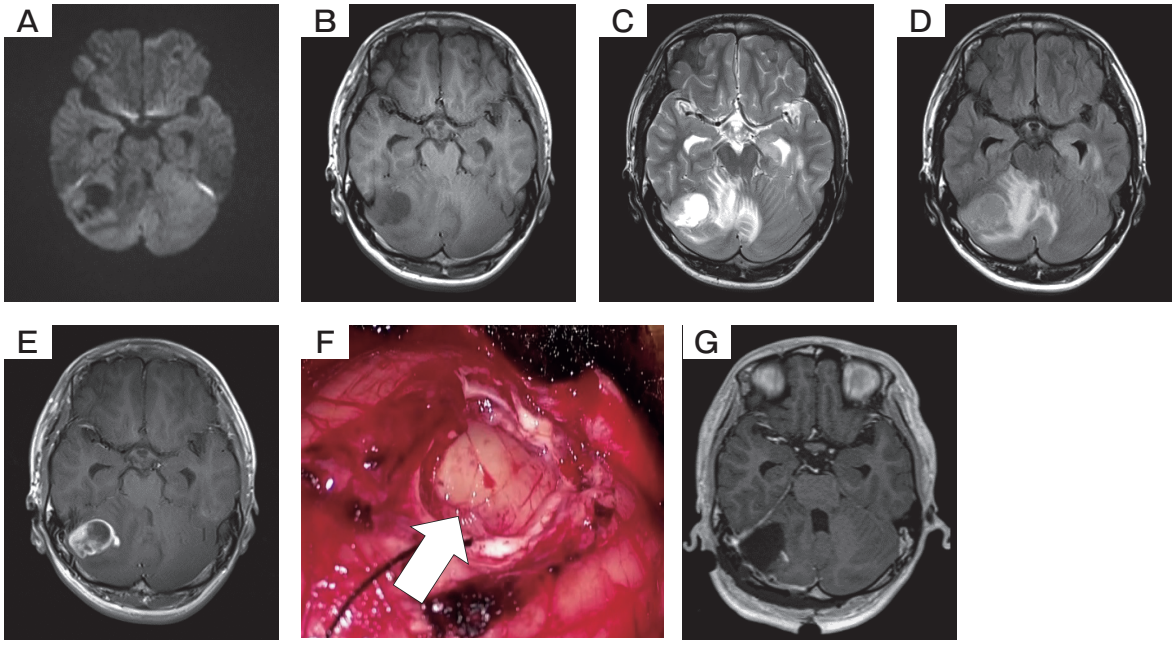


Fig. 1 Pre and postoperative MRI during the patient's first surgery. Head MRI before the initial surgery revealed a ring-like enhanced lesion in the right cerebellum (**A**, diffusion-weighted image; **B**, T1-weighted image; **C**, T2-weighted image; **D**, fluid-attenuated inversion recovery image; **E**, contrast-enhanced T1-weighted image). The fluid-attenuated inversion recovery image showed peritumoral edema (**D**). Intraoperative findings showed a brownish, soft, and internally hemorrhagic tumor. The border with the normal parenchyma was not discernible. An arrow indicates the tumor tissue lying underneath the normal cerebellar cortex (**F**). Postoperative MRI shows gross total resection of the contrast-enhanced lesion (**G**). MRI, magnetic resonance imaging.

performed. Postoperative MRI revealed gross total resection of the gadolinium-enhanced lesion (Fig. 1G).

Postoperative histological examination revealed a high number of small cells that were positive for GFAP and ATRX and negative for synaptophysin, p53, and IDH1R132H (Fig. 2A-G). Microvascular proliferation was also observed (Fig. 2A). The diagnosis was glioblastoma, NOS. The patient was subsequently treated with induction chemoradiotherapy consisting of proton-radiotherapy (60 Gy/30 fr) and concomitant daily oral temozolomide (75 mg/m^2). We chose proton-radiotherapy to protect critical structures. After induction therapy, he continued on temozolomide (150 mg/m^2 initially and 200 mg/m^2 thereafter) for 5 days every 28 days. At 13 months after his first operation, head MRI revealed a contrast-enhanced lesion at the bottom of the resected cavity (Fig. 3A-C). The patient underwent a second surgery using intraoperative MRI, and the enhanced lesion was completely removed (Fig. 3D-F). Hematoxylin and eosin showed a hypercellular tissue without microvascular proliferation or necrosis (Fig. 4A). Some tumor cells were positive for GFAP and synaptophysin, and most tumor cells were negative for NeuN and EMA. INI1 expression was preserved in the tumor cells, and the Ki-67 labeling index was approximately 60% (Fig. 4B-G). Direct sequencing revealed no hotspot alteration in *IDH1*, *IDH2*, or *BRAF*. These results suggested glioblastoma recurrence, but the positive synaptophysin results by immunohistochemistry were not compatible with the specimens from his first surgery. To make a precise diagnosis, we performed DNA methylation profiling of the initial tumor

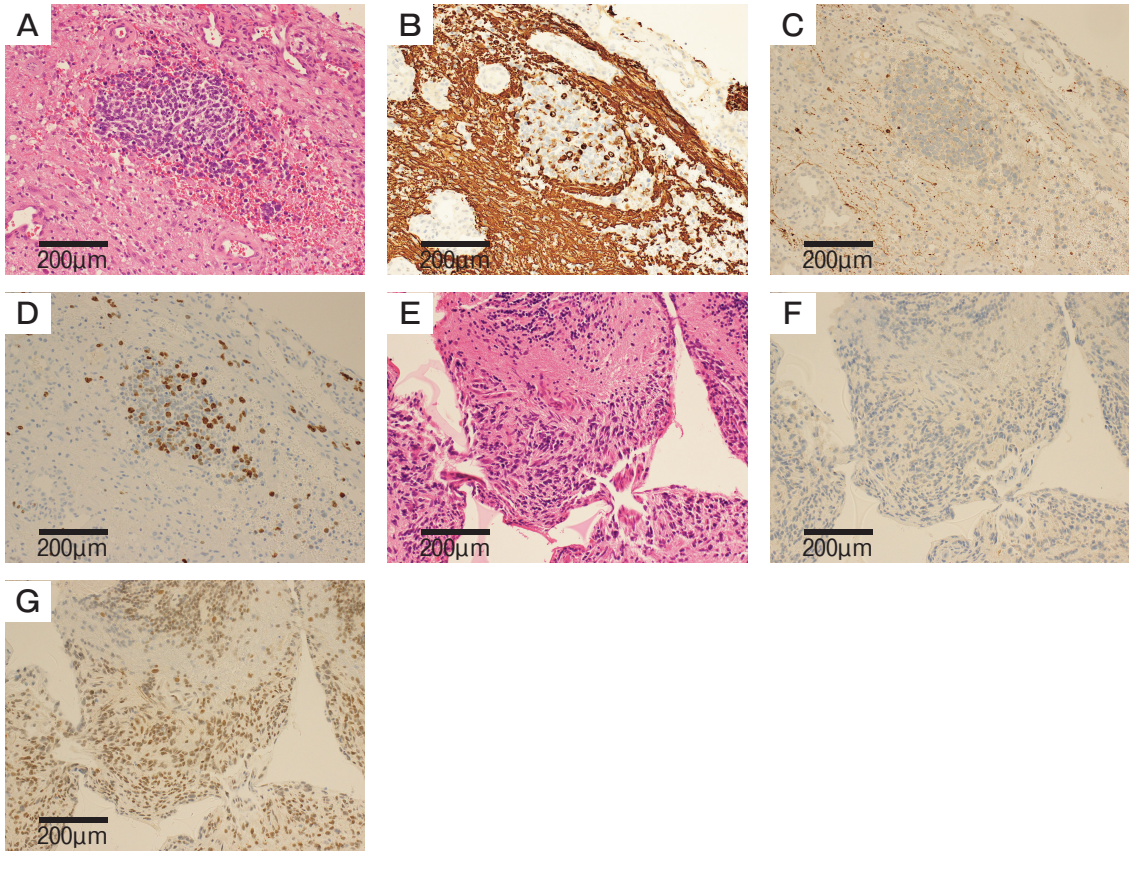


Fig. 2 Histopathological findings in the specimens from the patient's initial surgery. Hematoxylin and eosin staining showed hypervascularization in some areas and localized lesions with dense proliferation of small cells (**A**). The cells were GFAP-positive (**B**) and synaptophysin-negative (**C**) by IHC, and the Ki-67 labeling index was approximately 10% (**D**). Genetic analysis by IHC was conducted in another specimen from initial surgery. Tumor cells with large nuclei were observed in hematoxylin and eosin staining (**E**). IHC revealed no changes in IDH1R132H (**F**) and retention of ATRX (**G**) in tumor cells. IHC, immunohistochemistry.

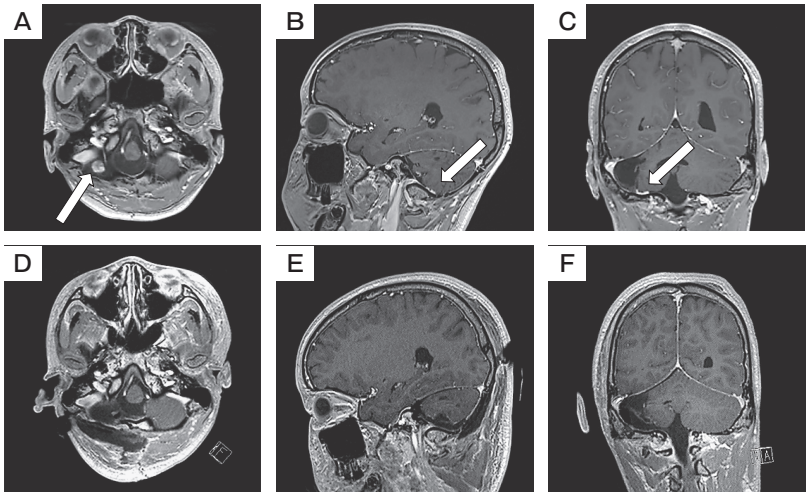


Fig. 3 Pre and postoperative contrast-enhanced MRI during the patient's second surgery. Head MRI before the second surgery showed the recurrence lesion at the bottom of the resected cavity (**A**, axial; **B**, sagittal; **C**, coronal). Arrows indicate the new tumor. Postoperative MRI showed the gross total resection of the new tumor (**D**, axial; **E**, sagittal; **F**, coronal).

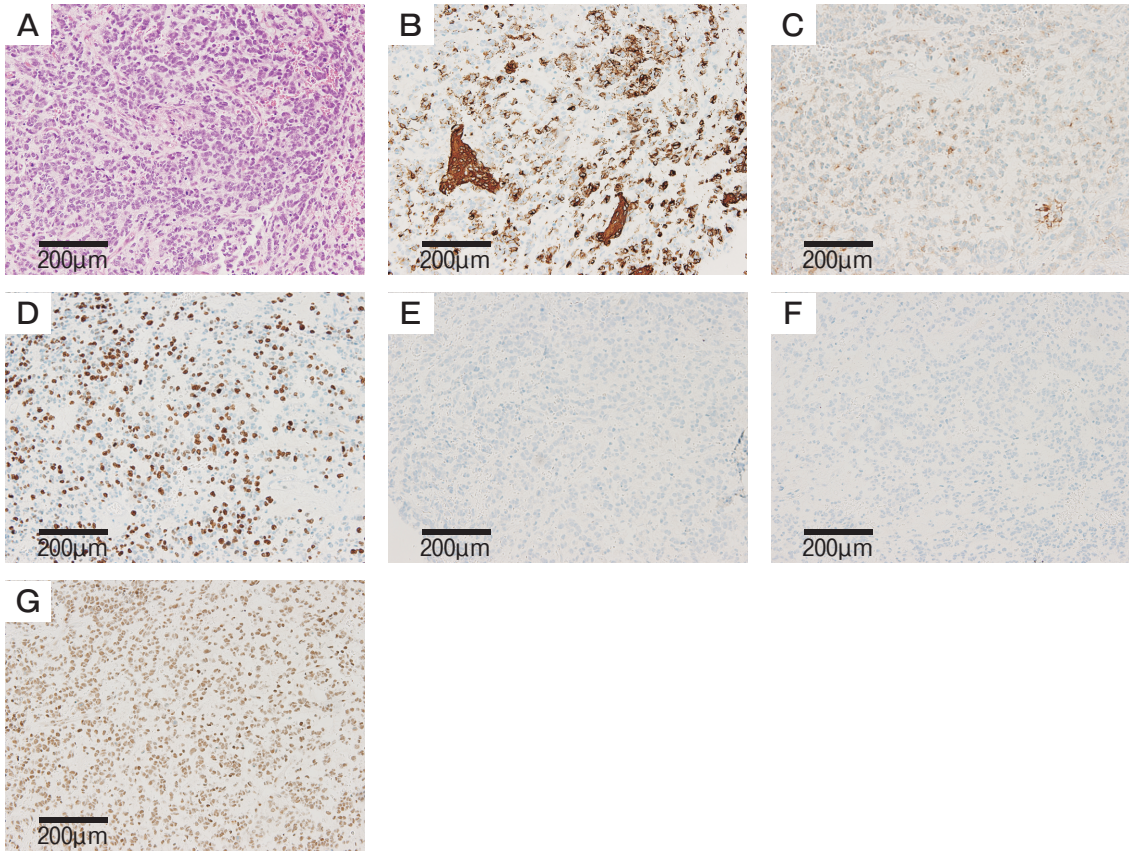


Fig. 4 Histopathological findings in specimens from the patient's second surgery Hematoxylin and eosin staining showed marked hypercellular neoplasm with round nuclei. Many mitotic figures were present, but no proliferation of vascular endothelial cells or necrosis was observed (**A**). Immunohistochemical staining showed focal GFAP (**B**) and synaptophysin (**C**) expression in tumor cells. Ki-67 labeling index was approximately 60% (**D**). Immunohistochemical staining results were negative for both NeuN (**E**) and EMA (**F**). INI1 expression was preserved in tumor cells (**G**).

and F1CDx profiling of the recurrent tumor. Infinium EPIC was performed using a frozen sample from the initial tumor, but DNA methylation profiling using both v11b4 and v12.5 revealed cerebellar hemisphere tissue due to insufficient tumor sampling (Fig. 5A). However, F1CDx of the recurrent tumor revealed *MYCN* amplification (Fig. 5B), and the tumor mutational burden was 1 Muts/Mb. Additionally, genetic alterations of *MITF*, *NOTCH3*, and *PPP2R2A* were detected as variants of unknown significance. No alteration was detected in *H3F3A* and *HIST1H3B*. Collectively, these results led us to diagnosis the recurrent tumor as a pediatric-type diffuse high-grade glioma, H3-wildtype and IDH-wildtype, in accordance with the current WHO classification, suggesting the utility of comprehensive genomic profiling.

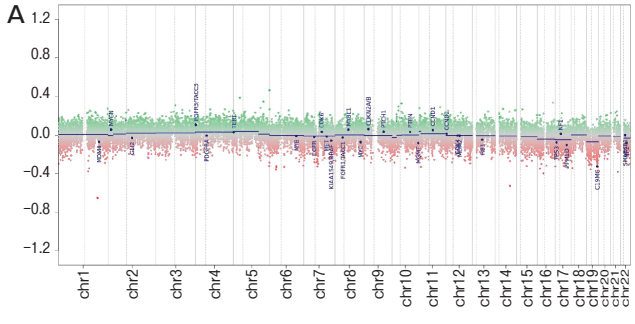
Discussion

Over the last decade, molecular analysis of pediatric gliomas has identified oncogenic driver genes: for instance, the RAS-mitogen-activated protein kinase (RAS/MAPK) alterations including *BRAF*, *NF1*, *FGFR*, *NTRK*, *RAF*, *ALK*, and *ROS1* as well as the non-RAS/MAPK alterations *MYB*, *MYBL1*, *IDH*, and *H3F3A* [9-12]. Thus, the diagnostic criteria for pediatric glioma now require a comprehensive genetic and epigenetic analysis.

Genome-wide DNA methylation profiling is the most powerful tool for making a precise diagnosis in CNS tumors [13], and it is essential or recommended in >50% of tumor entities in the current WHO classification. In pediatric diffuse glioma, six of seven tumor entities require genome-wide DNA methylation profiling. Capper *et al.* analyzed 1104 profiled samples using genome-wide DNA methylation, and 88% of the samples matched an established DNA methylation class with a calibrated classifier score of ≥0.9 [2]. Only 12% of tumors could not be assigned to a DNA methylation class. In another report with 502 cases, 26% of all cases were classified as having no methylation class match, but the authors mentioned that the methylation class and accompanying copy number variants plot could still be helpful for making a diagnosis [14]. For pediatric CNS tumors, Pickles *et al.* evaluated 306 samples, and the calibrated scores for 157 patients were less than 0.9 [5]. They further divided the precision of classifier predictions by the calibrated score range. For scores greater than 0.9, one in every 50 patients had an inaccurate diagnosis. However, for scores 0.7-0.9, one in every 14 patients was estimated to have an inaccurate diagnosis. Therefore, they concluded that cases with low calibrated scores should be considered with caution and used alongside other testing. In our case, we tested the frozen samples from initial tumors, but the diagnosis that was made using genome-wide DNA methylation profiling was "normal cerebellum". Because the tumor was small, our frozen sample might have had too few tumor cells to make a precise diagnosis using genome-wide DNA methylation profiling.

Recently, comprehensive genomic profiling tests for solid tumors such as F1CDx and NCC Oncopanel were approved by MHLW in Japan. Johnson *et al.* investigated the utility of F1CDx in 282 pediatric low- and high-grade gliomas [8,15]. In low-grade gliomas, genomic alterations such as *BRAF*, *NF1*, and *FGFR1* were identified in 119 of 125 cases (95.2 %). Additionally, among high-grade gliomas, 152 of 157 patients (96.8%) showed genetic alterations such as *TP53*, *H3F3A*, *ATRX*, *NF1*, and *PDGFRA*. Patients harboring fusion proteins including *KIAA1549-BRAF*, *QKI-RAF1*, *FGFR3-TACC3*, or hypermutated patients were also identified. Because

A



B

Point mutation	
MITF R393Q	VAF 0.869
NOTCH3 A1850T	VAF 0.5154
PPP2R2A Y71H	VAF 0.0592
Copy number alteration	
MYCN amplification	

Fig. 5 Results of Genome-wide DNA methylation profiling and FoundationOne CDx. Genome-wide DNA methylation profiling from the frozen tissue matched that of the normal cerebellum (**A**). FoundationOne CDx revealed *MYCN* amplification (**B**).

these genetic alterations are possible druggable targets in CNS tumor patients [16,17], F1CDx is considered useful for both diagnosis and treatment. In our case, we chose F1CDx for the recurrent tumor since previous reports showed its utility. *MYCN* amplification was identified, which led to a precise diagnosis in the recurrent tumor.

Diffuse pediatric-type high-grade glioma (pHGG), H3-wildtype and IDH-wildtype, is a diffuse glioma with histological features of malignancy that typically occurs in children, adolescents, or young adults, and it has wildtype *histone H3*, IDH1, and *IDH2* [18]. Three molecular subtypes are reported, including pHGG RTK1, pHGG RTK2, and pHGG MYCN, each with associated oncogenic drivers [19]. pHGG MYCN was previously diagnosed as primitive neuroectodermal tumor (PNET) or pontine glioma. Strum *et al.* identified 28 HGG-MYCN patients from 323 PNET cases [20], and Buczkowick *et al.* observed that 8% of pontine gliomas showed the MYCN subgroup [21]. pHGG MYCN is enriched in MYCN amplifications (approximately 50% of cases), and it is frequently co-amplified with the nearby *inhibitor of DNA binding 2 (ID2)* and the recurrent co-operating *TP53* mutation. pHGG MYCN occurs much more frequently in the supratentorial region (86%) compared with the infratentorial region (14%, mostly in brainstem) [19]; pHGG MYCN in the cerebellum is rare. Histologically, malignancy is obvious with high mitotic activity, and this is true for both supratentorial and pontine cases [22,23]. Tauziède-Espariat *et al.* reported that tumor cells co-expressed at least one glial and one neuronal marker including synaptophysin positivity in 3 of 5 cases [23]. pHGG MYCN has a poor prognosis among those three subtypes. The median overall survival (OS) of pHGG MYCN patients has been reported at 14 months, whereas the OS for pHGG RTK1 and RTK2 patients was 21 months and 44 months, respectively. Targeting MYCN is challenging due to its undruggable nature as a transcription factor and because it is also important in regulating developmental programs in healthy cells [24]. However, indirect inhibition of MYCN by drugs such as bromodomain-containing protein 4 (BRD4) inhibitors has shown efficacy in MYCN-amplified tumors including glioma; thus, further investigation may identify a treatment opportunity for pHGG MYCN [25,26].

In conclusion, we report the case of a patient with diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype, which was finally diagnosed using comprehensive genomic profiling. Because an accurate diagnosis leads to more precise treatment, comprehensive genetic and epigenetic analysis should be considered in pediatric patients with brain tumors.

Ethics approval. All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional research committee (IRB#1911-023) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from the patient's parents.

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