Pediatric Neurosurgery

Novel Insights from Clinical Practice

Pediatr Neurosurg 2020;55:319–325
DOI: 10.1159/000511672

Received: October 7, 2019
Accepted: September 12, 2020
Published online: November 23, 2020

Radiological and Immunostaining Characteristics of H3.3 G34R-Mutant Glioma: A Report of 3 Cases and Review of the Literature

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Established Facts

- The somatic mutation of the histone-H3 variant is associated with pediatric and adolescents and young adult tumors.
- H3.3 G34R/V mutation is predominantly identified in the supratentorial nonmidline tumors, but is not yet categorized as an entity in 2016 WHO CNS classification.

Novel Insights

- This report presented the radiological features of H3.3 G34R-mutant glioma: hyperintense on DWI, partial enhancement by gadolinium, mild peritumoral edema on T2WI/FLAIR, and high choline peak with lactate peak.
- This report also presented the immunohistochemical characteristics of H3.3 G34R-mutant glioma and the usefulness in the routine diagnostic test.

Keywords
H3.3 G34R mutation · Immunostaining · Diffusion-weighted imaging · Single-voxel proton magnetic resonance spectroscopy

Abstract
Introduction: H3.3 G34R/V mutation is predominantly identified in the supratentorial nonmidline tumors. However, this tumor is not yet categorized as an entity in 2016 WHO CNS classification. More information is necessary to further determine the characteristics of this tumor. ***Case Presentation:*** Three cases of adolescent hemispheric glioma were treated in our institution. All tumors showed the characteristics of huge tumor size with mild peritumoral edema on T2WI/FLAIR, hyperintense on DWI, and slight partial enhancement by gadolinium. The single-voxel proton MR spectroscopy revealed characteristics of high choline peak, marked decrease in N-acetyl aspartate peak, and small lactate peak. The histopathological diagnosis, based on 2007 WHO CNS classification, was high-grade glioma in 2 cases and a PNET. Immunostaining revealed that the tumor cells were positive against

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H3.3 G34R, H3K27me3, and p53 antibodies and negative against H3K27M, IDH1-R132H, ATRX, and Olig2 antibodies. Pyrosequencing analysis confirmed H3.3 G34R mutation, IDH-wildtype, and BRAF-wildtype. ***Conclusion:*** Radiological and immunostaining findings are characteristic in our 3 cases of H3.3 G34-mutant glioma. It is essential to consider H3.3 G34-mutant glioma as a differential diagnosis particularly in pediatric and adolescents and young adult hemispheric tumors.

Introduction

The somatic mutation of the histone 3 variant is associated with pediatric and adolescents and young adult (AYA) tumors [1]. Missense somatic mutations affecting histone H3F3A (H3.3) and HIST1H3B/C (H3.1) proteins are common in diffuse intrinsic pontine and thalamic gliomas as well as in a subset of cerebral hemispheric gliomas [2]. Furthermore, the K27M-mutation in H3.3 and H3.1 is defined as a new entity of "diffuse midline glioma, H3K27M-mutant" in the 2016 WHO Classification of Tumors of the Central Nervous System [3].

Further information is still required to understand the distinctive features of H3.3 G34-mutation, another type of H3-mutation in glioma. The 2016 WHO CNS classification does not include this entity yet. However, previous reports revealed that H3.3 G34-mutant glioma is predominantly found as a supratentorial nonmidline tumors [2, 4]. Moreover, this tumor is also found to account for 16.2% of cerebral hemispheric tumors in pediatric and AYA [5].

Regarding the difference in clinical course and biological behavior, H3.3 G34-mutant glioma should be defined as a separate entity from other IDH-wildtype astrocytic gliomas [6]. In this case report, we described some distinctive features of this rare tumor. We particularly highlighted additional information in regard to its radiological and immunostaining characteristics.

Case Report/Case Presentation

Case 1

A 13-year-old boy was transferred to our hospital with a progressive right hemiparesis. MRI showed a left frontal solid tumor with intratumoral hemorrhage. The tumor also had a daughter lesion in the left frontal lobe. T1WI (Fig. 1a) showed iso- to hypointensity. T2WI (Fig. 1b) and FLAIR (Fig. 1c) showed moderately hyperintense tumors. The tumors were poorly enhanced with gadolinium (Fig. 1e). Peritumoral edema was mild in spite of the huge tumor size on T2WI/FLAIR (Fig. 1b, c). In diffusion-weighted imaging (DWI), the main tumor revealed as hyperintense mass (Fig. 1d) with a minimum apparent diffusion coefficient (ADC) value of 0.625×10^{-3} mm^2/s. The tumor blood flow revealed to be low in arterial spin labeling-based perfusion-weighted imaging (PWI). Single-voxel proton MR spectroscopy (^{1}HMRS) was performed. The ROIs were placed by avoiding the hemorrhagic regions, which might influence these values. ^{1}HMRS with short echo time (TE) showed characteristic of high choline, disappearance of N-acetyl aspartate (NAA), and moderate lipid peak (Fig. 1g). ^{1}HMRS with intermediate TE exhibited small lactate peak (data not shown). CT demonstrated iso- to high-density masses without calcification (Fig. 1f).

A gross total surgical removal of the tumor was obtained on postoperative MRI. The routine hematoxylin-eosin (HE) staining unveiled small and round basophilic tumor cells with scant cytoplasm. The tumor showed poorly differentiated cells of neuronal lineage with palisading necrosis (Fig. 1h). Immunostaining demonstrated positivity for H3.3 G34R (Fig. 1i), H3K27me3, and p53 antibodies (Fig. 1j). The tumor was negative for IDH1-R132H, H3K27M, Olig2 (Fig. 1k), and ATRX antibodies (Fig. 1l). Ki-67 labeling index (LI) was approximately 90% in hot spot areas. Fluorescence in situ hybridization (FISH) analysis showed no deletion in the 1p/19q chromosome. Pyrosequencing analysis also confirmed the status of H3.3 G34R mutation, IDH-wildtype, and BRAF-wildtype. The tumor was initially diagnosed as primitive neuroectodermal tumor (PNET). However, according to the updated references and molecular analysis, the diagnosis was then revised as glioblastoma, H3F3A G34R-mutant. The patient was treated with chemoradiotherapy. Unfortunately, the tumor recurred in between the primary and the daughter tumor site. The patient died 36 months after the initial surgery.

Case 2

A 19-year-old female was referred to our hospital with dysesthesia and generalized convulsion. MRI revealed a right parietal tumor. T1WI showed iso- to hypointense signaled mass. T2WI and FLAIR showed moderate hyperintense tumor. The tumor was only partially enhanced with gadolinium. As in case 1, the peritumoral edema was mild despite the tumor size on T2WI/FLAIR. In DWI, the tumor revealed as hyperintense mass with a minimum ADC value of 0.788×10^{-3} mm^2/s. The tumor blood flow revealed to be low in arterial spin labeling-based PWI (data not shown). ^{1}HMRS revealed a characteristic of high choline peak, marked decrease in NAA peak, slight lactate peak, and no lipid peak (data not shown). CT scan demonstrated an isodensity mass without calcification (data not shown).

A gross total surgical removal of the tumor was obtained on postoperative MRI. The routine HE staining unveiled the tumor cells that demonstrated characteristics of astrocytes' origin. They clustered around small- to medium-sized blood vessels as well as showing patchy and infiltrative growth pattern in cerebral parenchymal tissue. Immunostaining demonstrated positivity for H3.3 G34R, H3K27me3, and p53 antibodies. The tumor was negative for IDH1-R132H, H3K27M, Olig2, and ATRX antibodies. Ki-67 LI was approximately 40% in hot spot areas. FISH analysis showed no deletion in the 1p/19q chromosome. Pyrosequencing analysis also confirmed the status of H3.3 G34R mutation, IDH-wildtype, and BRAF-wildtype. The tumor was initially diagnosed as high-grade glioma. Thus, based on the molecular analysis, the diagnosis

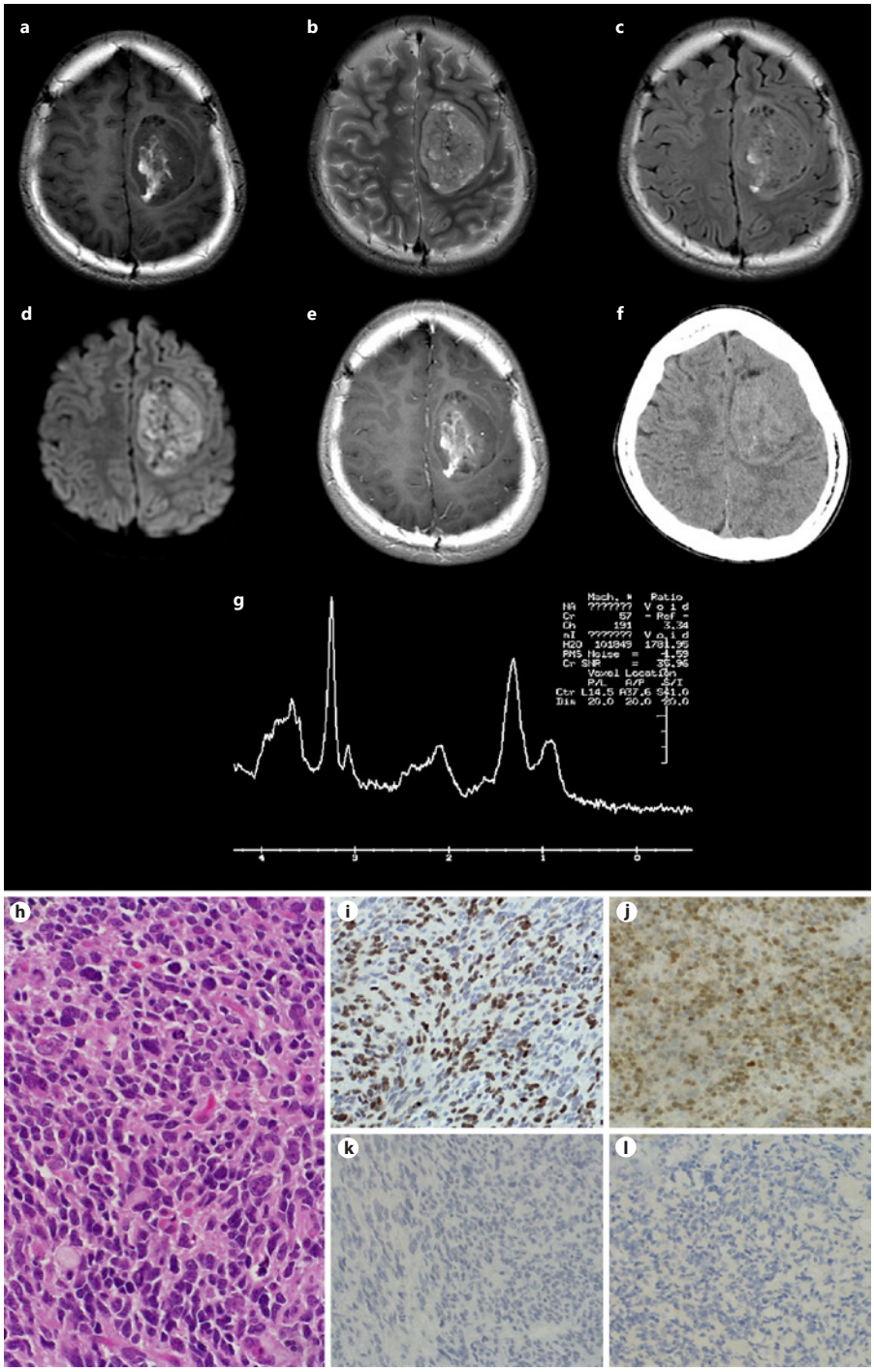

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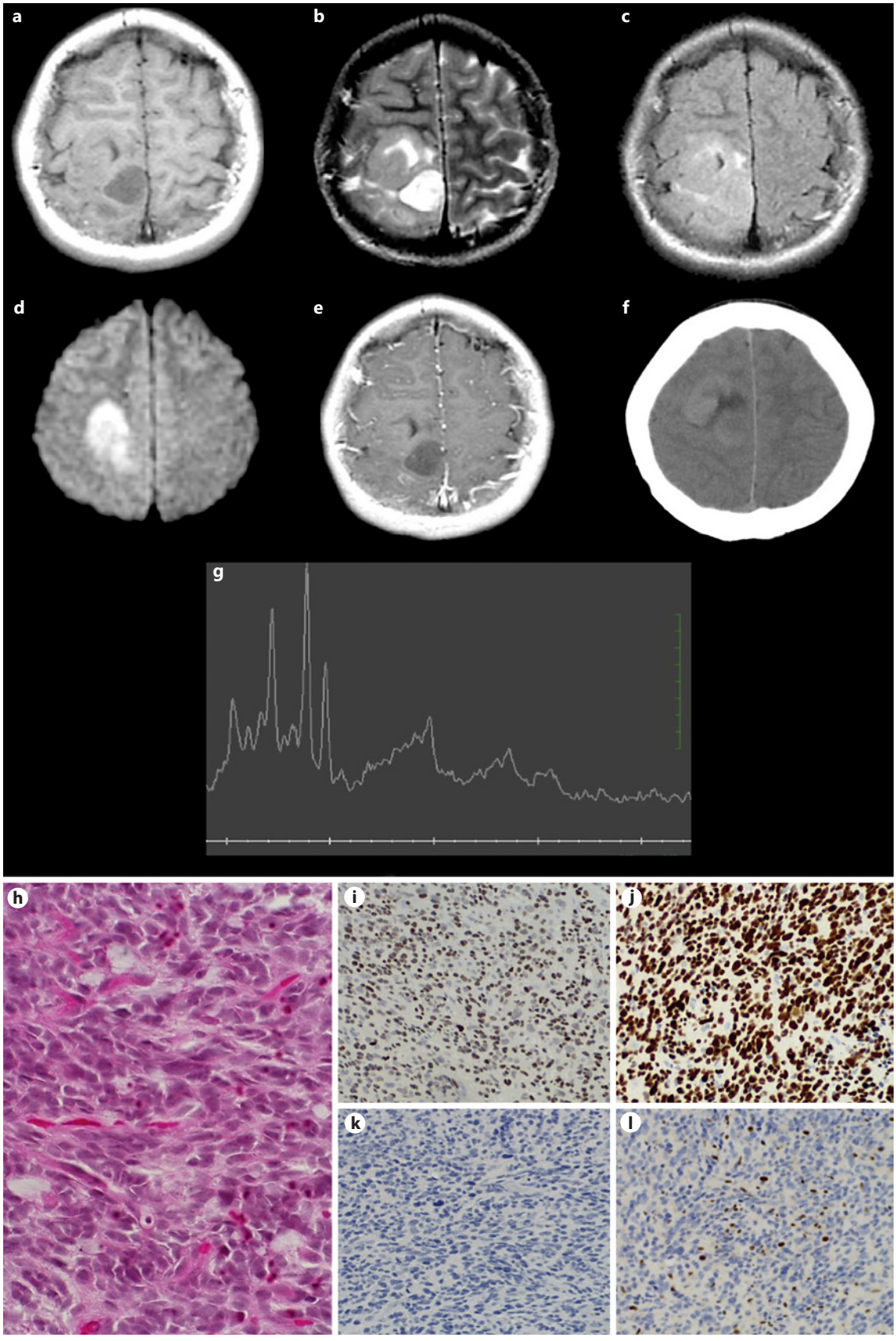
Fig. 1. Case 1: MRI showed a left frontal tumor with iso-to hypointense on T1WI (**a**), moderately hyperintense with solid component on T2WI (**b**) and FLAIR (**c**), and hyperintense on DWI with an ADC value of 0.625×10^{-3} mm^2/s (**d**). **e** The tumor was poorly enhanced with gadolinium. **f** CT scan showed iso-to high-density masses without calcification. **g** ^{1}HMRS with short TE demonstrated a characteristic of high choline, disappearance of NAA, and moderate lipid peak. **h** HE staining demonstrated poorly differentiated basophilic tumor cells with neuronal differentiation. The tumor was positive for H3.3 G34R (**i**) and p53 (**j**) immunostaining and negative for Olig2 (**k**) and ATRX (**l**). DWI, diffusion-weighted imaging; ADC, apparent diffusion coefficient; ^{1}HMRS, single-voxel proton MR spectroscopy; TE, echo time; NAA, *N*-acetyl aspartate; HE, hematoxylin-eosin.

was revised as high-grade glioma, H3F3A G34R-mutant. The patient was treated with chemo- and radiotherapy. She was free of recurrence for 18 months since the initial surgery.

Case 3

A 15-year-old female presenting with a progressive left hemiparalysis was referred to our hospital. MRI revealed a right parieto-occipital tumor with partial enhancement. T1WI (Fig. 2a) showed an iso- to hypointense mass. T2WI (Fig. 2b) and FLAIR (Fig. 2c) showed moderate hyperintense tumor. The tumor was only partially enhanced with gadolinium (Fig. 2e). As we found in the previous cases, the peritumoral edema was also mild irrespective of the tumor size on T2WI/FLAIR. In DWI, the tumor revealed as hyperintense mass (Fig. 2d) with a minimum ADC value of 0.810×10^{-3} mm^2/s. ^{1}HMRS revealed a characteristic of high choline peak, marked decrease in NAA peak, slight lactate peak, and



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no lipid peak (Fig. 2g). CT scan demonstrated an iso- to high-density mass with no calcification (Fig. 2f).

The tumor was surgically excised partially. The routine HE staining unveiled the pleomorphic cells with hyperchromatic anaplastic nuclei and scant-to-moderate amount of cytoplasm (Fig. 2h). Immunostaining demonstrated positivity for H3.3 G34R (Fig. 2i), H3K27me3, and p53 antibodies (Fig. 2j). The tumor was negative for IDH1-R132H, H3K27M, Olig2 (Fig. 2k), and ATRX antibodies (Fig. 2l). Ki-67 LI was approximately 37% in hot spot areas. FISH analysis showed no deletion in the 1p/19q chromosome. Pyrosequencing analysis also confirmed the status of H3.3 G34R mutation, IDH-wildtype, and BRAF-wildtype. Therefore, the initial diagnosis of glioblastoma was then revised as glioblastoma, H3F3A G34R-mutant. The patient was also treated with chemo- and radiotherapy. Unfortunately, the tumor recurred in a form of local invasion and the patient died 15 months after the initial surgery. Immunostaining and molecular analysis results are summarized in Table 1.

Discussion/Conclusion

In this report, we presented a detailed radiological characteristic of H3.3 G34R-mutant glioma using various diagnostic tools, and there were similar characteristics in our cases: the slight or partial contrast enhancement with gadolinium, mild peritumoral edema on T2WI/FLAIR, and high intensity on DWI. DWI displays the diffusion of water molecules, and the ADC values indicate the cellularity of a mass [7–9]. Therefore, our finding of DWI hyperintense may be associated with high cellularity in H3.3 G34R-mutant glioma. On ^{1}HMRS, a high choline peak was also shown in all cases. The radiological pattern also indicated that this tumor growth has a tendency to infiltrate the surrounding parenchyma.

A definite histopathological diagnosis of H3.3 G34-mutated glioma is unattainable only from routine HE analysis. H3.3 G34R/V glioma unveiled various histological phenotypes, such as glioblastoma, central nervous system primitive neuroectodermal tumors (CNS-PNET), and astroblastoma [10–12]. In regard to the molecular classification of CNS-PNET, Sturm et al. [12] reported that this tumor did not form any distinct characteristic. Hence, it is mostly classified into the various other well-defined CNS tumor entities. Based on the study by Sturm et al. [12], 17 of 323 (5%) CNS-PNET cases were reclassified into H3F3A G34-mutant high-grade glioma. In one of our cases (case 1), the diagnosis was similarly changed from PNET to glioblastoma, H3F3A G34R-mutant, based on molecular findings. Based on this finding, we realized that it is necessary to consider H3.3 G34R/V-mutated glioma as a differential diagnosis in supratentorial brain tumor, particularly in pediatric and AYA.

In regard that histone mutation and IDH mutation are mutually exclusive, our result on IDH and H3.3 G34R/V-mutations were also consistent in both immunostaining and pyrosequencing analyses. The use of immunostaining against IDH1-R132H antibody is widely accepted and applied [13]. Haque et al. [14] discovered that immunostaining for H3.3 G34R/V-mutant in glioma is also highly concordant with the genotype analysis. Our cases confirmed that the use of immunohistochemistry staining to detect H3.3 G34R-mutations is more efficient, compared

Table 1. Immunostaining and molecular analysis results

	IDH1/2	BRAF	H3.3 G34R	H3 K27M	H3 K27me3	Olig2	ATRX	p53	Ki-67 LI, %
Case 1	Wildtype†	Wildtype†	+†	−†	+	−	−	+	90
Case 2	Wildtype†	Wildtype†	+†	−†	+	−	−	+	40
Case 3	Wildtype†	Wildtype†	+†	−†	+	−	−	+	37

Positive cutoff values of ATRX and p53 were 10% nuclear staining. LI, labeling index. † Pyrosequencing analysis also confirmed the status.

Fig. 2. Case 3: MRI showed a right parietal tumor with iso- to hypointense with mild peritumoral edema on T1WI (**a**), hyperintense on T2WI (**b**) and FLAIR (**c**), and hyperintense on DWI with an ADC value of 0.810×10^{-3} mm^2/s (**d**). **e** The tumor was poorly enhanced with gadolinium. **f** CT scan showed an iso- to high-density mass with no calcification. **g** ^{1}HMRS revealed a characteristic of high choline peak, marked decrease in NAA peak, slight lactate peak and no lipid peak. **h** HE staining demonstrated pleomorphic cells with hyperchromatic anaplastic nuclei and scant-to-moderate amount of cytoplasm. The tumor was positive for H3.3 G34R (**i**) and p53 (**j**) immunostaining and negative for Olig2 (**k**) and ATRX (**l**). DWI, diffusion-weighted imaging; ADC, apparent diffusion coefficient; ^{1}HMRS, single-voxel proton MR spectroscopy; TE, echo time; NAA, *N*-acetyl aspartate; HE, hematoxylin-eosin.

to other molecular analysis, concerning the routine diagnostic test. Consequently, this also confirmed the promising feature of H3.3 G34R immunostaining as a diagnosis tool of this rare tumor.

With immunostaining, we also found that the Olig2 negativity is intriguing in regard to H3.3 G34R-mutant glioma. It is well acknowledged that Olig2 is expressed in some subsets of stem and progenitor cells in normal brain, diffuse gliomas (WHO grade II/III), and some genetic subtypes of glioblastoma [15]. However, based on an analysis of gene expression, Olig2 gene expression was significantly lower in H3.3 G34-mutated tumors [4]. Therefore, Olig2 negativity in IDH-wildtype glioma is potentially useful to anticipate H3.3 G34R-mutation in hemispheric glioma, particularly in pediatric and AYA populations. In addition, other immunohistochemistry staining results in our cases also gave more perspective. The ATRX and p53 immunostaining positive cutoff values were both 10% nuclear staining as described previously [16]. All of our cases showed consistent results with previous reports of ATRX-negative immunostaining expressions [11, 17]. High Ki-67 LI in our cases confirmed the DWI hyperintensity which may be associated with high cellularity in this glioma.

In conclusion, we observed radiological features of hyperintense on DWI, partial enhancement by gadolinium, mild peritumoral edema on T2WI/FLAIR, and high choline peak with small lactate peak, as well as Olig2 negativity, in IDH-wildtype glioma as promising characteristics for H3.3 G34R-mutant glioma. It is essential to consider H3.3 G34-mutant glioma as a differential diagnosis regarding these characteristics particularly in pediatric and AYA hemispheric tumors.

Acknowledgements

We would like to express our special thanks to Dr. Yoshiko Nakano and Dr. Koichi Ichimura, Division of Brain Tumor Translational Research, National Cancer Center Research Institute, for their valuable advice and the technical support and Dr. Junko Hirato, Department of Pathology, Gunma University Hospital, for the central diagnosis.

Statement of Ethics

This case report was approved by Hiroshima University Hospital institutional review board, which waived the need for informed consent for publication.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

There is no funding source to declare.

Author Contributions

Shumpei Onishi and Fumiyuki Yamasaki interpreted the patient data regarding the radiological images and molecular findings and are the major contributors in writing the manuscript. Vishwa Jeet Amatya and Yukio Takeshima performed the histopathological examination. Kazuhiko Sugiyama and Kaoru Kurisu contributed to the systemic interpretation of the patients' data. Shumpei Onishi wrote the initial draft of the manuscript. Vega Karlowee assisted in the preparation of the manuscript. All authors read and approved the final manuscript.

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