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Clinical study

Adult diffuse midline gliomas: Clinical, radiological, and genetic characteristics

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ABSTRACT

Diffuse midline gliomas (DMGs) are a diffuse glioma subtype arising from midline brain structures. It is predominantly a disease of childhood; however, it can also occur in adults. Adult DMG has not been previously well described. The aim of this study was to define the characteristics of adult DMG. We described and analyzed the clinical, radiological, and genetic alterations of 9 adult DMGs and compared them with those of 257 non-midline adult high-grade IDH-WT gliomas. The median age of all patients was 38-years old (23–68-years). Most common symptoms were headache, motor/sensory deficit, ataxia, cranial nerve deficit, and confusion. Tumor locations were brainstem (44.5%), thalamus (22.2%), pineal region (22.2%), spinal cord (22.2%), and cerebellum (11.1%). Six-patients (66.7%) were H3 K27M-WT and three (33.3%) were H3 K27M-mutant. In addition to H3 K27M mutations, *TP53* gene (55.5%), *CDKN2A/B* and *TERTp* (33.3%), *PDGFRA* (33.3%), *PIK3CA, PTEN, KDR, NF1*, and *MYC* (22.2%) were the most frequently mutated genes. Neither *IDH1/IDH2* nor *EGFR* alterations were present. Compared to non-midline high-grade glioma, adult DMG patients were younger (38 vs 61 years, $p < 0.001$) and lacked *EGFR*-alterations (0/9 vs 123/257, $p = 0.004$). The median survival of DMG and non-midline high-grade gliomas was 19 and 18 months respectively ($p = 0.964$). Our data support that adult DMGs have different oncogenic drivers compared to non-midline high-grade gliomas. Regardless of H3 K27M mutation status, neither of the nine adult DMG cases demonstrated *IDH1/IDH2* or *EGFR* alterations. Larger multi-institutional studies are needed to further characterize the biology of this rare type of diffuse glioma in adults.

1. Introduction

Diffuse midline gliomas typically arise in midline structures such as the brainstem (midbrain, pons, and medulla), thalamus, spinal cord, pineal region, cerebellum, hypothalamus, or third ventricle [1]. The 2016 World Health Organization (WHO) classification of the central nervous system tumors introduced the diagnosis "diffuse midline glioma (DMG), H3 K27M–mutant" as a new entity corresponding to WHO grade IV, regardless of the histologic characteristics [2]. Even though this entity is predominantly a pediatric disease, it can also occur in adults [3–7]. Remarkably, DMG H3 K27M in adults has been consistently reported to have a better prognosis compared to pediatric patients [5,7–12]. A recent study demonstrated that DMG H3 K27M tumors in the brainstem conferred a poor prognosis compared to DMG H3 wildtype (WT) in adults, however, pilocytic astrocytomas and gangliogliomas were considered in the H3 WT group, which could have biased the results [6]. Moreover, a study comparing DMG H3 K27M mutant to DMG H3 WT regardless of their location did not observe survival differences between these groups [3].

Genetic characterization of DMG H3 K27M in the pediatric population has demonstrated that genetic drivers are different from adult lobar gliomas; showing increased frequency in *TP53, ACVR, CCND1-3, and CDK4/CDK6* mutations [9,10,13,14]. Moreover, it has been demonstrated that genetic alterations commonly reported in high-grade lobar gliomas in adults (*EGFR, TERTp, IDH1/IDH2)* are absent or rare in pediatric DMG H3 K27M tumors [1,9,13]. Likewise, the decrease expression in immunohistochem-

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istry examination of *EGFR, TERTp,* and *IDH1/IDH2* has also been described in adults DMG H3 K27M tumors [3,5,7,14–17].

Even though the clinical and genetic characteristics of DMG H3 K27M mutant and WT have been extensively defined in the pediatric population [8–10,13,18,19] and the clinical characteristics of adult DMG have been recently reported [4–7], the biology and behavior of H3 K27M-mutant adult DMG remain a subject of debate. In particular, whether or not these tumors exhibit similar biology and behavior as their pediatric counterparts remain unclear. Furthermore, a comprehensive genetic characterization of these tumors in adults have yet to be described.

The aim of this study was to report the clinical, radiological, pathological, and molecular characteristics of adult H3 K27M mutant and WT DMG and compare them with gliomas without *IDH1/IDH2* and H3 K27M mutations occurring outside midline structures.

2. Material and methods

2.1. Patients and tumor samples

We retrospectively identified adult DMG patients using an institutional glioma database. Cases were included in the study if they met the following criteria: 1) diagnosis of diffuse glioma; 2) >18-years of age at the time of presentation; 3) midline location (brainstem, thalamus, cerebellum, pineal gland, or spinal cord); 4) profiled by next-generation sequencing.

Clinical data were collected from the electronic medical records of Memorial Hermann Hospital and compiled into a REDCap database. These included age, gender, Karnofsky performance status (KPS), histologic diagnosis, tumor location, volumetric extent of resection, treatment strategy, recurrence, and survival. Tumors were classified by a board-certified neuropathologist following the 2016 WHO Classification of Tumors of the Central Nervous System. This study was approved by the institutional review board of the University of Texas Health Science Center at Houston and Memorial Hermann Hospital, Houston, TX.

A cohort of 257 non-midline adult high-grade IDH-WT gliomas (glioblastoma (GBM) and anaplastic astrocytoma), without H3 K27M mutations, was used to compare genetic alterations and outcomes between groups.

2.2. Targeted sequencing

Tumor samples were analyzed for genetic alterations by a targeted next-generation sequencing assay (NGS) interrogating 315 genes and 28 gene rearrangements (FoundationOne CDx®, Foundation Medicine Inc., Cambridge, MA, USA) [20,21]. The FoundationOne® assay was performed in a clinical laboratory improvement amendments (CLIA)-certified laboratory, as previously described [22,23].

2.3. Statistical analyses

Clinical, demographic, and frequency of genetic alterations in adult DMG and non-midline IDH-WT diffuse glioma were evaluated by Fisher's exact test. Overall survival between adult DMG and non-midline high-grade gliomas was calculated from the date of diagnosis to the date of death or last available follow-up. Kaplan-Meier method was used to plot survival curves and the statistical significance was examined by the Log-rank test. A p-value of <0.05 as statistically significant. Data analysis was performed in EZR (1.40) [24] and GraphPad Prism (version 8.2.1, La Jolla California USA).

3. Results

3.1. Clinico-demographic characteristics of adult DMG

A total of 564 patients between 2004 and 2019 were identified and 9 patients met the inclusion criteria (supplementary Fig. 1). There were 6 males and 3 females; interestingly, all males were H3 K27M WT, meanwhile, all females were H3 K27M mutant. The median age was 38-years old (Range: 23–68 years); the H3 WT patients' average age was 43.3 years vs 31.3 years for H3 K27M mutant cases. The most common presenting symptom was headache (66%), followed by motor/sensory deficit, (55.5%), ataxia (33.3%), cranial nerve deficit (22.2%), and altered mental status/-confusion (11.1%). The median KPS was 70.

Among the 9 cases, the majority were located in the brainstem (44.5%) followed by thalamus (22.2%), pineal gland (22.2%), spinal cord (22.2%), and cerebellum (11.1%). Contrast enhancement was present in 6/8 (75%) of the cases and radiographic hydrocephalus in 44.5%. (Figs. 1 and 2). Due to the eloquent location of the tumors, resection was only performed in (45.5%) of patients (subtotal or needle biopsy was done in 88.9% of patients) and most patients were treated according to the Stupp protocol [25].

All patients were histologically diagnosed with high-grade glioma IDH-WT GBM, except patient 9 (diffuse astrocytoma grade II). All H3 K27M WT patients were GBM IDH-WT meanwhile, in the H3 K27M mutant cases, patients were diagnosed as GBM, anaplastic astrocytoma, and diffuse astrocytoma. However, all H3 K27M mutant patients were cataloged as DMG, H3 K27M mutant, Grade IV, according to the 2016 WHO classification [2]. Table 1 summarizes the clinical, radiological, and pathological characteristics of our cohort.

The Fischer-exact test comparison of the clinical characteristic between adult DMG and non-midline adult high-grade gliomas indicated that patients with DMGs were younger (median 38 vs 61 years old, $p < 0.001$). Moreover, this comparison was sustained when the 6 DMG H3 K27M WT were compared against their non-midline counterparts (median 43.3 vs 61 years old, $p < 0.001$). Table 2 and Supplementary Table 1.

3.2. Genetic alterations

NGS analysis identified 49 genetic alterations involving 31 genes. Alterations designated as variants of unknown significance (VUS) were not taken into account in the analysis. The median number of mutations detected per patient were 6 (range 3–8). Details of the genetic alterations and VUS are presented in Supplementary Table 2. As demonstrated in Fig. 3 and Table 1, 3/9 (33.3%) patients showed the H3 K27M mutation.

Fig. 3 demonstrates the most common mutations in adult DMG found in our cohort. In addition to H3 K27M mutations, the most common alterations in the adult DMG cohort were in *TP53* (55.5%), which was present in 66.7% of the H3 K27M mutant patients and 50% of the H3 K27M WT patients. *CDKN2A/B* and *TERTp* alterations were present in 33.3% of the patients, however, none of the H3 K27M mutant patients had alterations in these genes, compared to 50% of H3 K27M WT patients. Other common mutations identified in our cohort included *PDGFRA* in 33.3% of patients (33.3% in H3 K27M mutant and WT patients), *PTEN* in 22.2% of patients (0% in H3 K27M mutant and 33.3% in H3 K27M WT patients), *KDR, PIK3CA, NF1,* and *MYC,* all mutated in 22.2% of patients (33.3% in H3 K27M mutant and 16.7% in H3 K27M WT patients). Importantly no *IDH1/IDH2* or *EGFR* alterations were found in the DMG cohort.

The Fischer-exact test comparison of the most common genetic alterations between adult DMG and non-midline adult high-grade

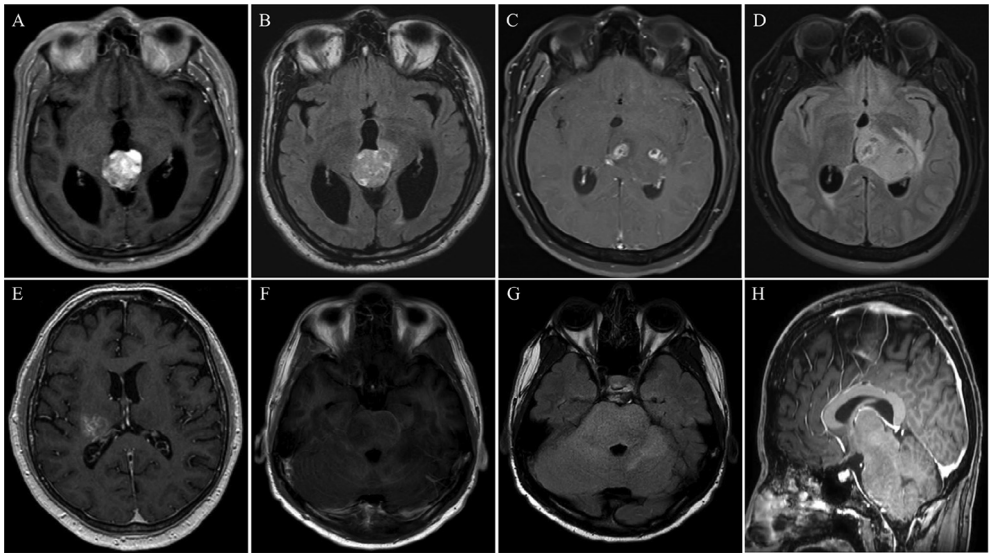

Fig. 1. Adult diffuse midline glioma H3 K27M wildtype MRI imaging. Patient 1, pineal/posterior third ventricular region Glioblastoma IDH-WT (A. Axial T1-post contrast. B. Axial T2 FLAIR). Patient 3, focal enhancing left thalamic Glioblastoma IDH-WT (C. Axial T1-post contrast. D. Axial T2 FLAIR). Patient 5, focal enhancing, right thalamic Glioblastoma IDH-WT (E. Axial T1-post contrast). Patient 6, non-enhancing pontine and cerebellar peduncle Glioblastoma IDH-WT (F. Axial T1-post contrast. G. Axial T2 FLAIR. H. Sagittal T1-post contrast).

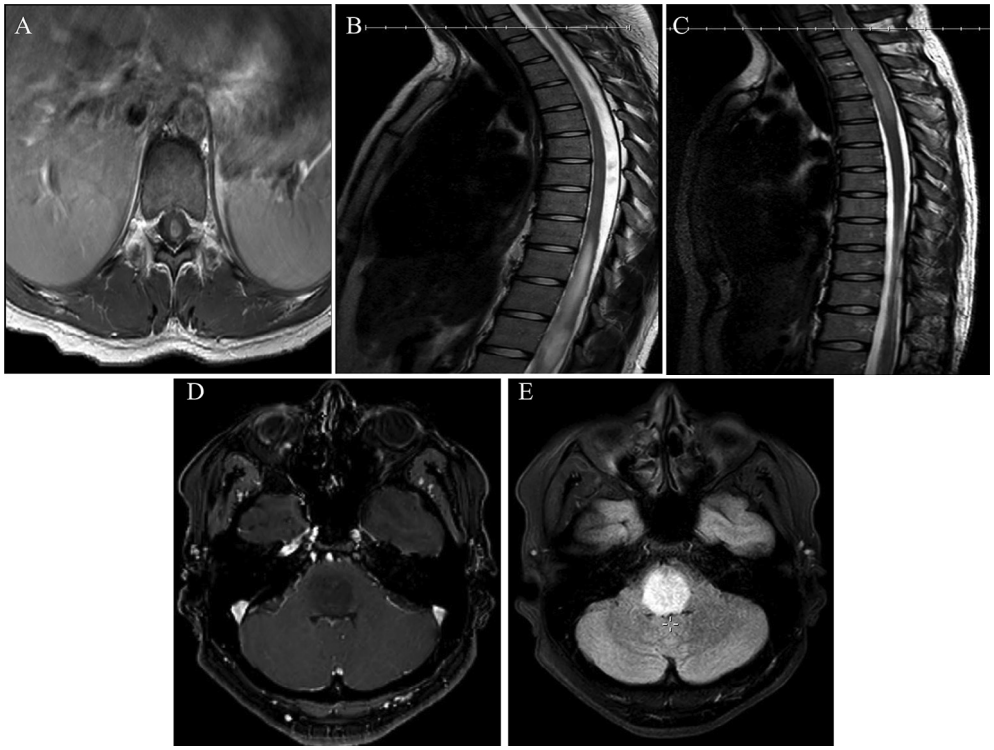

Fig. 2. Adult diffuse midline glioma H3 K27M mutant MRI imaging. Patient 7, lower thoracic spinal cord Diffuse Midline Glioma H3 K27M mutant (A. Axial T1-post contrast. B. Sagittal T2). Patient 9: upper thoracic spinal cord Diffuse Midline Glioma H3 K27M mutant (C. Sagittal T2) Patient 8, non-enhancing pontine Diffuse Midline Glioma H3 K27M mutant (D. Axial T1-post contrast. E. Axial T2 FLAIR).

glioma IDH-WT demonstrated increased alterations of *MYC* in the DMG group (2/9 vs 3/257, $p < 0.01$). Also, we found that DMG had a lower frequency of alterations in *EGFR* and *CDKN2A/B* genes compared to non-midline IDH-WT adult high-grade gliomas (0/9 vs 123/257, $p = 0.004$ and 3/9 vs 182/257$p = 0.02$, respectively). Importantly, *EGFR* differences remained significant in the comparison between the H3 K27M WT subgroup and the non-midline cohort (0/6 vs 123/257, $p = 0.03$). Table 3 and Supplementary Table 3.

3.3. Prognosis and survival

Survival differences between DMG (n = 9) and non-midline high-grade gliomas (n = 257) were evaluated with no observed difference in overall survival (OS) in the log-rank test (DMG; 19-months vs. non-midline high-grade gliomas; 18 months, $p = 0.964$). Fig. 4. The longest survival corresponds to patient 9 with a DMG, H3 K27M mutant in the upper thoracic cord, who survived 34.8-months. Interestingly, this patient would have been diag-

Table 1
Demographic, clinical, and radiological characteristics of adult diffuse midline glioma patients. M: male. F: female. A: Asian. W: white. H: Hispanic. HA: headaches. N/V: nausea and vomits. KPS: Karnofsky performance status. GBM: glioblastoma. AA: anaplastic astrocytoma. DA: Diffuse Astrocytoma. HCP: Hydrocephalus. EOR: extent of resection. STR: sub-total resection. GTR: gross-total resection. TMZ: temozolomide. XRT: radiotherapy. TTF: tumor treating-fields. Rec: recurrence. Bev: bevacizumab. PFS: progression-free survival. OS: overall survival. NA: Not available. *Patient did not have a recurrence. ǂ Patient is alive.

No	Age	Sex	Symptoms	Location	KPS	Histologic Diagnosis	MRI	HCP	EOR	Treatment	*H3F3A* (H3) status	PFS	OS
1	54	M	HA, N/V, Confusion, Ataxia	Brainstem, Pineal Gland	70	GBM	Heterogeneous enhancement	Present	STR	TMZ, XRT	None	11.7	12.1ǂ
2	24	M	HA	Pineal Gland	80	GBM	Minimal enhancement	Present	Biopsy	XRT Rec: Re-resection, TMZ.	None	7.6	19.0
3	40	M	HA, Motor and Sensory deficit	Thalamus, Brainstem	70	GBM	Focal enhancement	Present	Biopsy	TMZ, XRT Rec: Bev.	None	6.0	8.1 ǂ
4	50	M	HA, N/V, Ataxia, dysmetria, dysarthria	Cerebellum	60	GBM	NA	Absent	GTR	TMZ, XRT	None	3.3*	6.4
5	68	M	Motor and Sensory deficit	Frontal, Thalamus	70	GBM	Minimal enhancement	Absent	Biopsy	TMZ, XRT Rec: Bev, TTF.	None	13.9	20.1
6	24	M	HA, ataxia, Dysphagia, diplopia, hemiparesis, hemiplegia.	Brainstem, Cerebellum	50	GBM	No enhancement, exofitic lesion	Absent	STR	CCNU, TTF, XRT.	None	7.2*	7.2 ǂ
7	23	F	Hemiplegia	Spinal Cord	50	GBM	Focal enhancement	Absent	Biopsy	TMZ, Bev, XRT.	K27M	9.0*	9.3
8	38	F	HA, N/V, hemiparesis	Brainstem	70	AA	No enhancement	Present	Biopsy	TMZ, XRT Rec: Bev.	K27M	9.4	16.7 ǂ
9	33	F	Motor and Sensory deficit	Spinal Cord	60	DA	Focal enhancement	Absent	STR	TMZ, XRT. Rec: TMZ, Bev, CPT-11, XRT.	K27M	11.8	34.8

Table 2
Adult Diffuse Midline Glioma (n = 9) and non-midline High-grade glioma IDH/ H3 K27M WT (n = 257) clinical characteristics differences. The Fischer-exact test was used for all categorical variables. A *p*-value of < 0.05 was determined as significant (highlighted on green). KPS: Karnofsky-performance status. EOR: extent of resection. TMZ: temozolomide. TTF: tumor-treating fields. XRT: radiotherapy. Dx: At diagnosis. SRS: stereotactic radiosurgery. DMG: diffuse midline glioma. GTR: gross-total resection. WT: wild type.

Adult Diffuse Midline Glioma and non-midline High-grade glioma IDH/ H3 K27M WT clinical characteristics differences.				
Location		DMG	Non-DMG	Fisher *p*-value
Age	>55	1	190	**0.000209**
	<55	8	67	
Ethnicity	Caucasian	5	182	0.58
	Non-Caucasian	4	75	
Gender	Male	6	152	0.74
	Female	3	105	
KPS	<80	6	158	1
	> or =80	3	99	
EOR	GTR	1	86	0.28
	Non GTR	8	171	
TMZ as 1st line therapy	Yes	7	235	0.20
	No	2	22	
TTF as 1st line therapy	Yes	1	29	1
	No	8	228	
XRT as 1st line therapy	Yes	9	232	1
	No	0	25	
Other Chemotherapy as 1st line therapy	Yes	0	13	1
	No	9	249	
Bevacizumab as 1st line therapy	Yes	1	23	1
	No	8	234	
Salvage Bevacizumab	Yes	4	115	1
	No	2	65	
Salvage CPT-11	Yes	1	59	0.67
	No	5	121	
Salvage XRT	Yes	1	43	1
	No	5	137	
Salvage SRS	Yes	0	60	0.176
	No	5	120	
Salvage TMZ	Yes	2	76	1
	No	4	104	
Salvage TTF	Yes	1	56	0.67
	No	5	124	
Other Salvage Chemotherapy	Yes	0	19	1
	No	5	161	

Table 3
Adult Diffuse Midline Glioma (n = 9) and non-midline High-grade glioma IDH/ H3 K27M WT (n = 257) most common genetic alterations. The Fischer-exact test was utilized for all categorical variables. A *p*-value of <0.05 was determined as significant (highlighted on green). DMG: diffuse midline glioma. WT: wild type. Mut: mutant or altered. Del: deleted.

Adult Diffuse Midline Glioma and non-midline High-grade glioma IDH/ H3 K27M WT genetic differences				
Location		DMG	Non-DMG	Fisher *p*-value
H3F3A	WT	6	257	**0.000027**
	Mut	3	0	
TERTp	WT	6	91	0.078
	Mut	3	166	
CDKN2A/B	WT	6	75	**0.025**
	Del	3	182	
EGFR	WT	9	134	**0.0042**
	Mut	0	123	
PTEN	WT	7	129	0.17
	Mut	2	128	
PIK3CA	WT	7	227	0.30
	Mut	2	30	
MDM2/4	WT	8	220	1
	Mut	1	37	
PDGFRA	WT	6	220	0.14
	Mut	3	37	
PIK3C2B	WT	8	243	0.41
	Mut	1	14	
TP53	WT	3	178	0.15
	Mut	6	79	
CDK4/6	WT	8	218	1
	Mut	1	39	
GLI1	WT	8	249	0.27
	Mut	1	8	
MYC	WT	7	254	**0.0097**
	Mut	2	3	
NF1	WT	7	218	0.63
	Mut	2	39	
TET2	WT	8	249	0.27
	Mut	1	8	
RB1	WT	8	232	1
	Mut	1	25	
KIT	WT	9	233	1
	Mut	0	24	
KDR	WT	7	235	0.19
	Mut	2	22	
SETD2	WT	8	246	0.34
	Mut	1	11	
CCND1-3	WT	8	255	**0.010**
	Mut	1	2	
PTPN11	WT	9	248	1
	Mut	0	9	
FGFR3	WT	9	249	1
	Mut	0	8	
ATRX	WT	8	250	0.24
	Mut	1	7	
BRAF	WT	9	251	1
	Mut	0	6	
ASXL1	WT	9	251	1
	Mut	0	6	
HGF	WT	9	251	1
	Mut	0	6	
SOX2	WT	9	251	1
	Mut	0	6	

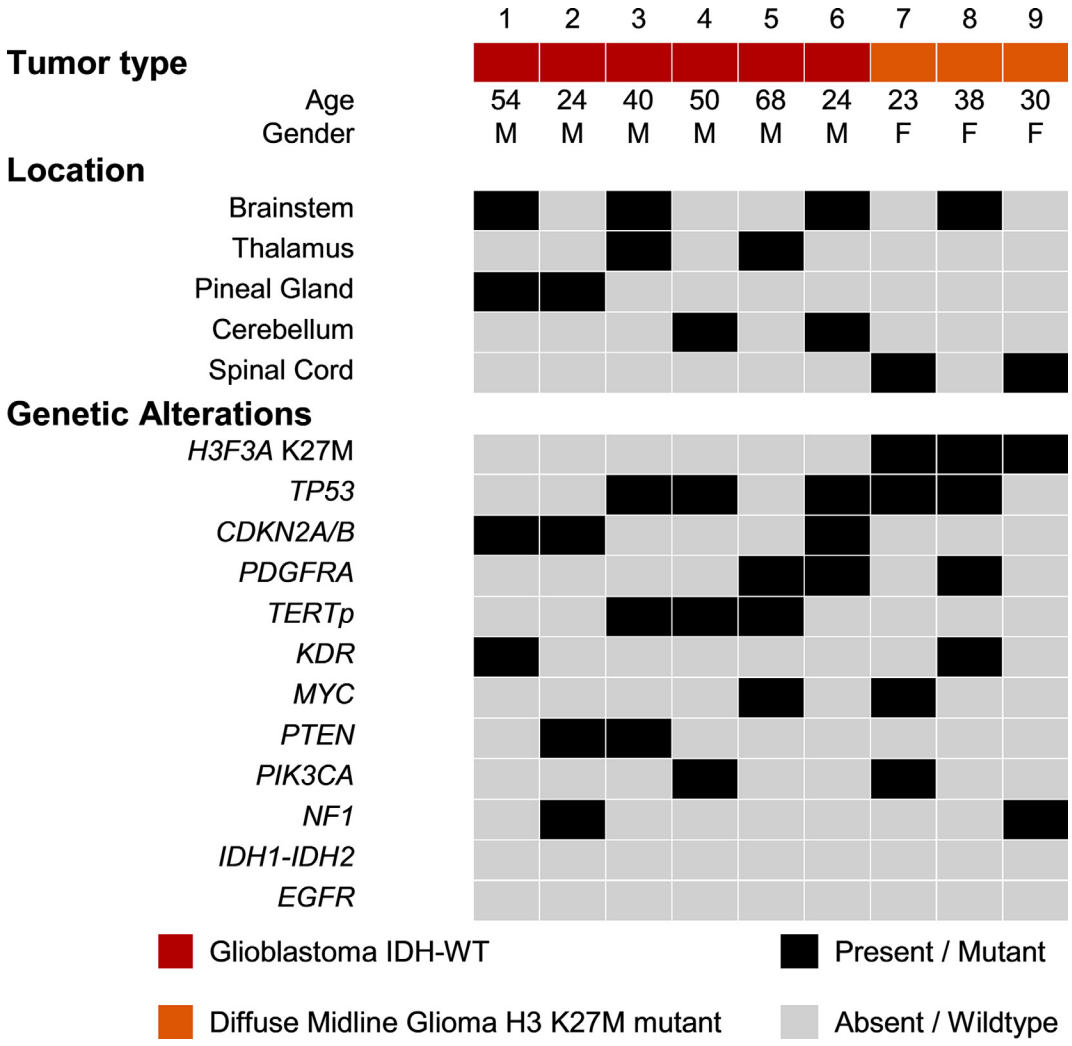

Fig. 3. Histologic diagnosis, location, and genetic alterations in patients with Adult Diffuse Midline Glioma.

nosed by histology (2007 WHO CNS tumor classification) as low-grade glioma (diffuse astrocytoma, IDH-WT, WHO grade II).

4. Discussion

Adult DMGs are aggressive tumors of the CNS which have been poorly characterized due to their rarity. Most insights into DMGs come from pediatric and young adult patients, and it is unclear whether their behavior is comparable to those occurring in adults [4–7]. Few studies have described the genetic profiles of H3 K27M mutant and WT adult DMG [14–17,26]. This could be due in part to the rareness of these tumors and the fact that biopsy is typically performed for diagnosis, with limited tissue available for molecular analysis. Furthermore, the prognosis of H3 K27M mutation in adult DMGs is unclear, with contradicting studies in terms of survival compared to H3 WT DMG and non-midline gliomas [5–7,14–16]. In the current study, we report the clinicopathological, radiological, and genetic characteristics of 9 adult DMG.

The incidence of gliomas involving midline structures in our adult population was ~5%; however, after excluding patients with lobar tumors that invaded midline structures it was ~4%. This is lower compared to prior studies evaluating the incidence of radiographically determined adult diffuse midline gliomas, which have been identified in 19% of gliomas [27]. The difference between our study and this prior report of the DMG incidence in adults are explained by the criteria utilized to catalog tumors as DMG. In the present study, we evaluated patients histologically diagnosed rather than based on imaging. In addition, the aforementioned study included DMG tumors located in the basal ganglia, midline cortex, and corpus callosum and grade I tumors, which could explain their higher percentage of tumors defined as DMG. Moreover, a recent study evaluating adult DMG utilizing a similar definition than our study identified an incidence of 4% in a cohort of 2649 gliomas [26]. Furthermore, the National Cancer Institute Center for Cancer Research estimates that around 447 adults are diagnosed with DMG [28]. Considering that the most recent report of the CBRTUS estimates that infiltrating gliomas in adults will be comprised of ~18,050 in 2020 [28], we estimate that DMG constitutes approximately 3% of adult infiltrating gliomas, a similar percentage compared to our series. However, further investigation is needed to identify the incidence of adult DMG H3 K27M mutant and DMG H3 K27M WT.

Age differences between DMG and non-midline high-grade gliomas in our cohort appear to be in line with prior studies with DMG patients being younger [6,7]. Importantly, when the cohort was subdivided by the H3 K27M mutation, mutant patients were younger than their WT counterparts (30.3 vs 43.3 years old) (Table 1). Patients presented with symptoms related to their eloquent location, and hydrocephalus was frequently observed. As these tumors are typically unresectable, either biopsy or STR was

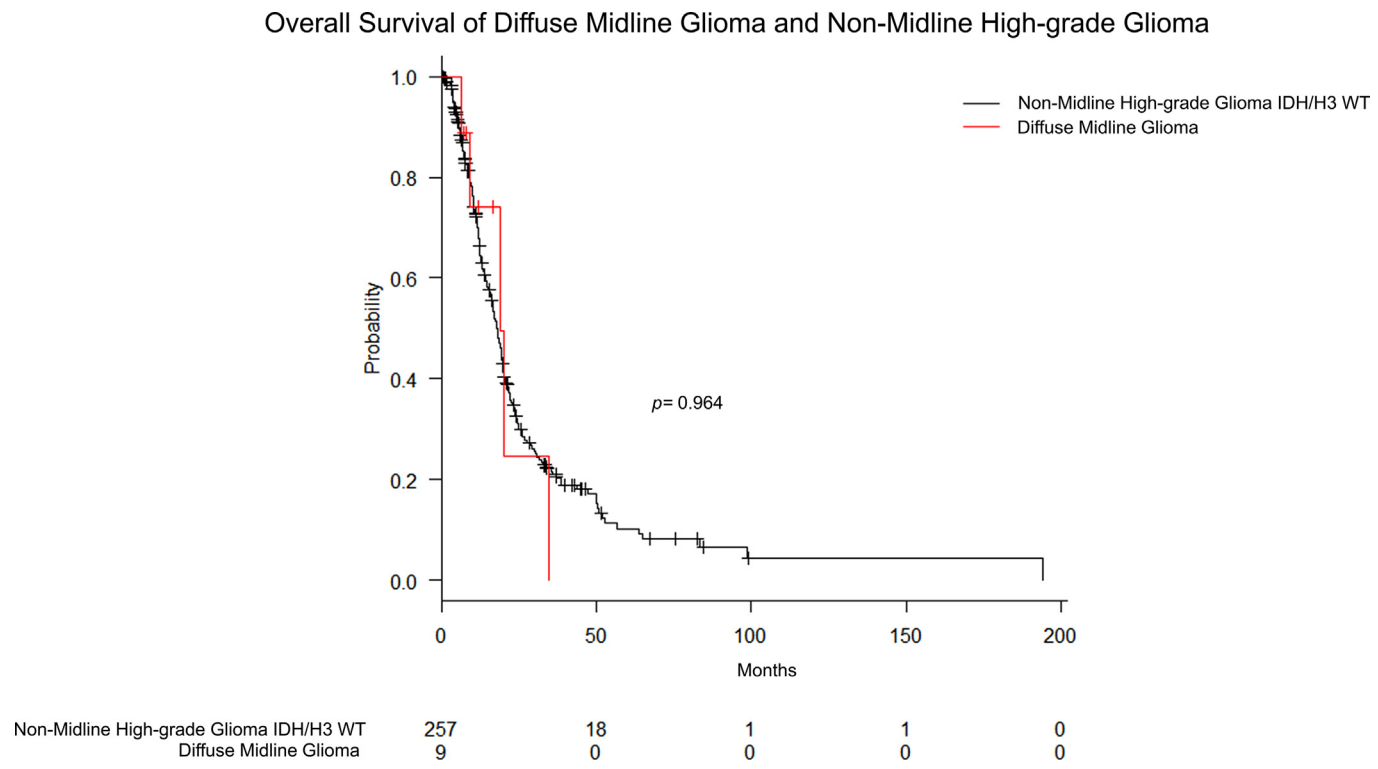

Fig. 4. Kaplan Meier survival curves between adult diffuse midline glioma and non-midline high-grade glioma IDH/H3 WT (median 19-months vs 18-months, p = 0.964).

performed in the majority of cases. The lack of cytoreductive surgery could potentially contribute to poor outcomes in these cases [15], however, previous studies in thalamic GBM have not demonstrated worse outcomes in patients undergoing biopsy rather than resection [29]. DMGs patients that were H3 K27M WT were diagnosed histologically as GBM and comprise 67% of our cohort, meanwhile, 1/3 (33.3%) of H3 K27M mutant patients were diagnosed as GBM histologically. In accordance with other studies that have observed a frequency of 37.5% to 66% of H3 K27M mutation in adult DMG, we observed a frequency of 33.3% for the H3 K27M mutation in our DMG cohort [7,15–17,30].

As expected, *IDH1/IDH2* and *EGFR* alterations were absent in H3 K27M mutated patients [3,5,7,14–17]. Remarkably, both alterations were also not present in the H3 K27M WT adult DMG patients, which has been previously described [17]. A reduced frequency of *CDKN2A/B, TERTp,* and *PTEN* mutations was observed when comparing adult DMG with non-midline high-grade gliomas. Moreover, our data suggest that *PIK3CA, PDGFRA,* and *MYC* alterations might be more common in adult DMGs. Table 2. Even though these differences were not statistically significant in our study, the differences in the frequency of genetic alterations in these genes have been noted in prior studies [14–17]. Importantly, *EGFR* was observed to be reduced in adult DMG H3 K27M WT when compared to non-midline high-grade gliomas. Supplementary Table 2.

In our cohort H3 K27M mutant DMG showed a similar molecular pattern as previously described in pediatric and young adult patients, adding to the notion that this tumor entity is genetically similar regardless of the age of presentation. Moreover, the well-known differences in oncogenic drivers in pediatric DMG H3 K27M mutant appear to remain in adults [3,5,7,10,13–17,19]. Notably, DMG H3 K27M WT appear to have a different pattern of genetic alterations compared to non-midline high-grade gliomas, with the absence of *EGFR* and *IDH1/IDH2* alterations, which is in concordance with previous reports [17].

Survival data in adult DMG is currently contradictory [4–7,15–17,27,29]. A prior study evaluating brainstem gliomas observed that H3 K27M mutated tumors had significantly shorter survival than H3 WT tumors (9-months vs. not reached, respectively) [6]. However, the study included lower-grade tumors which are known to have improved survival such as pilocytic astrocytoma. A recent study evaluating adult DMG identified that patients harboring H3 K27M mutations had better survival than midline high-grade glioma K27M WT patients [27]. However, this study included tumors located in the basal ganglia, midline cortex, and corpus callosum. Another study discussed that H3 K27M confers a worse prognosis compared to IDH/ H3 K27M WT midline and non-midline glioma, however, no statistical significance was observed [7]. Moreover, their study also included non-diffuse lower-grade tumors, such as pilocytic astrocytoma and ganglioglioma. Contrary to the pediatric literature [5,9–12], multiple studies on DMG H3 K27M mutation in adults have reported that this mutation does not confer a worse survival compared to non-midline high-grade gliomas [3,7,15]. This issue has been raised by the recent second update of c-IMPACT NOW, where the diagnosis of DMG H3 K27M requires the tumor to be diffuse, besides being midline, glioma, and H3K27M mutant [31]. Taking this into consideration, when adult DMG is compared to non-midline high-grade glioma, no differences in survival are identified [7,17,29]. In our study, comparison between DMG and non-midline high-grade glioma also showed no differences in OS (median 19-months vs 18-months).

There are several limitations in the current study, particularly the retrospective nature as well as the limited number of cases. We also acknowledge that strong conclusions cannot be drawn from our cohort. However, taking into consideration the rareness of this disease and the current lack of large-scale studies incorporating genomic analysis, our study adds to the body of literature in this subtype of diffuse gliomas. We further examined the differences between DMG subtypes, H3 K27M mutant, H3 K27M WT, and non-midline high-grade gliomas. In concordance with previously published studies, we found that H3 K27M mutant gliomas could be considered a "pediatric-like" tumor with different oncogenic drivers. Notably, DMG and non-midline adult high-grade

gliomas appeared to have a dismal prognosis, without a clear relevance of H3 K27M-mutation as a prognostic marker. Larger multi-institutional studies are needed to better characterize the molecular alterations of these rare tumors, to expand our understanding of both adult DMG H3 K27M mutant and WT tumors.

5. Conclusions

Our data support that adult DMGs have different oncogenic drivers compared to non-midline high-grade gliomas. Regardless of H3 K27M mutation status, neither of the nine adult DMG cases demonstrated IDH or *EGFR* alterations. Larger multi-institutional studies are needed to further elucidate the biology of this rare diffuse glioma in adults.

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Conflict of interest

The authors have no duality or conflicts of interest to declare.

CRediT authorship contribution statement

Antonio Dono: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Takeshi Takayasu:** Data curation, Writing - review & editing. **Leomar Y. Ballester:** Conceptualization, Supervision, Writing - review & editing. **Yoshua Esquenazi:** Conceptualization, Supervision, Writing - review & editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jocn.2020.10.005.

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