Original Article

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Clinicopathological characterization and prognostic factors of H3 K27M-altered diffuse midline gliomas: A real-world cohort study

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SUMMARY: Diffuse midline glioma (DMG) is a high-grade malignant brain tumor. The 5-year survival rate for DMG remains close to zero. It is a rare and highly aggressive tumor, which is more common in children than in adults. We here report our retrospective study of 210 cases of DMG. Surgical tumor samples were analyzed by routine histopathology and immunohistochemistry for H3 K27M, ATRX, p53, OLIG2 and Ki-67. The most frequent anatomic locations in patients were the thalamus and brainstem. During follow-up, 169/210 patients (80.5%) died from the disease, with a median survival time of 11 months (range: 0 to 87 months). Kaplan-Meier analysis indicated that the prognosis of adult patients was better than that of the pediatric cohort ($P = 0.006$). Multivariable Cox regression identified male sex, spinal cord tumor location, and a Ki-67 index of ≤ 10% as independent favorable prognostic factors. Also, meta-analysis indicated that the prognosis of adult patients was better than that of the pediatric cohort ($P = 0.002$), patients with brainstem tumors had a worse prognosis compared to those with tumors in other locations ($P = 0.003$). We highlight the aggressive nature of DMG and the critical role of clinical and molecular markers in prognosis. These findings demonstrate substantial clinicopathological heterogeneity in H3 K27M-altered DMG and support the combined consideration of age, tumor location, and proliferative activity in future prognostic stratification. Prospective multicenter validation and more comprehensive molecular profiling are required.

Keywords: diffuse midline glioma, prognostic factors, clinicopathological characteristics

1. Introduction

H3 K27-altered diffuse midline gliomas (DMG) are aggressive and currently incurable intracranial gliomas, characterized by high levels of intrinsic resistance to multiple therapies (*1*). In 2021, the 5th edition of the WHO Classification of Tumors of the Central Nervous System redefined these tumors as "Diffuse midline glioma, H3 K27-altered," reflecting the recognition of multiple underlying mechanisms beyond mutation. This classification incorporates various molecular alterations, primarily involving changes in H3 K27, TP53, ACVR1, PDGFRA, EGFR, and EZHIP (*2*). H3 K27-altered DMG predominantly occurs in pediatric populations and is infrequently observed in adults, comprising approximately 50% of all pediatric high-grade gliomas (HGGs). This malignancy is associated with a high mortality rate, characterized by a median overall survival (OS) of less than one year (*3*). In contrast, adult DMG represents a much smaller and less well-characterized subgroup, and its clinical behavior, molecular profile, and prognostic determinants remain incompletely understood. Despite increasing recognition of H3 K27-altered DMG, most published studies have focused on relatively small cohorts, predominantly pediatric populations, or isolated molecular features. Consequently, the integrated relationships among clinical presentation, histopathological characteristics, molecular markers, and patient outcomes remain insufficiently characterized, particularly in real-world clinical practice. This limitation hampers accurate prognostic stratification and the development of individualized management strategies.

The incidence of DMG is notably low, which often constrains the comprehensive analysis of its clinical characteristics and molecular mechanisms due to the limited number of cases. Consequently, it is crucial to conduct an in-depth examination of the key molecular

features of DMG to elucidate its pathogenesis and identify therapeutic targets, utilizing analyses derived from extensive case reports. Numerous therapeutic targets have been identified, with several undergoing rigorous preclinical investigation. Research has been conducted on targeting epigenetic modifications (*4*) (such as PRC2 and HDACs), growth factor receptors (*5*) (including EGFR and IGF1R), cell cycle checkpoints (*6*) (such as CDK4/6 and WEE1), DNA damage repair mechanisms (*7*) (including PARP and GD2), and immunotherapy. However, the efficacy of these targets remains uncertain. Importantly, the prognostic relevance of clinical and molecular factors may vary across patient subgroups, particularly between pediatric and adult DMG, potentially influencing therapeutic responsiveness and clinical trial stratification (*8,9*). Understanding the independent prognostic factors of DMG is essential for further exploration of personalized treatment efficacy targeting specific factors. This knowledge can guide the development of tailored therapeutic strategies for different patient populations.

In this study, we performed a comprehensive clinicopathological evaluation of 210 patients with H3 K27M-altered DMG using real-world clinical data collected from a large tertiary referral center. We systematically integrated clinical characteristics, histopathological findings, immunohistochemical profiles, survival analyses, and an external meta-analysis to comprehensively evaluate prognostic determinants of this rare tumor. By including both pediatric and adult patients within a single unified framework, this study provides clinically relevant evidence regarding age-related differences, molecular prognostic markers, and risk stratification, thereby helping bridge existing knowledge gaps and providing practical information for future personalized management of DMG.

2. Materials and Methods

2.1. Patient cohort

We retrospectively reviewed 210 patients with H3 K27M-altered DMG who were diagnosed and treated at Xiangya Hospital of Central South University between September 2016 and July 2023. Overall survival was defined as the interval from diagnosis to death or the last follow-up. The inclusion criteria were: 1. The tumor was located in a midline position. 2. The imaging and pathological diagnosis were consistent with DMG. The histopathologic findings were reviewed by 3 neuropathologists. This study retrospectively analyzed the clinical, imaging, pathological, and molecular characteristics of these patients. Among them, there were 102 male patients (48.6%) and 108 female patients (51.4%), with 122 adult patients (58.1%) and 88 pediatric patients (41.9%) (patients aged ≤ 18 years were classified as pediatric patients, whereas those aged > 18 years were classified as adults). A total of 199 patients (94.8%) underwent surgical resection, while the remaining 11 patients (5.2%) underwent stereotactic biopsy. All procedures involving human participants were conducted in accordance with the Declaration of Helsinki. The study was approved by the Medical Ethics Committee of Xiangya Hospital, Central South University (Approval number: 202210233). Informed consent was obtained from each patient or the patient's legal guardian for the conducted research. This study was reported in line with the PROCESS Guideline (*10*).

2.2. Immunohistochemistry

IHC was carried out as described previously (*11,12*). Tumor tissues were fixed in formalin, dehydrated, embedded in paraffin, and sectioned into 3–5 μm slices for hematoxylin and eosin (H&E) and immunohistochemical (IHC) staining. After deparaffinization and rehydration, antigen retrieval was performed using sodium citrate buffer. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H_2O_2). To minimize non-specific staining, sections were blocked with 10% goat serum at 37°C for 30 minutes. Subsequently, the sections were incubated with diluted primary antibodies overnight at 4°C (ZSGB-BIO, Beijing, China; GFAP: ZA-0529; Ki-67: ZM-0167; OLIG2: ZM-0169; ATRX: ZA-0016; p53: ZM-0408; H3 K27M: ZA-0321). The following day, biotin-labeled IgG secondary antibodies were applied for 30 minutes at room temperature. Staining was developed using DAB, followed by counterstaining with hematoxylin. Finally, slides were covered by coverslips using gelatin (*13*). Immunoreactivity was considered positive when nuclear staining of Ki-67, OLIG2, ATRX, p53, or H3 K27M was observed in tumor cells, whereas GFAP positivity was defined by cytoplasmic staining. Nuclear staining in tumor cells was assessed for both intensity and proportion. Tumors were considered p53-positive if ≥ 10% of tumor nuclei showed strong staining, and p53-negative if < 10% or absent staining. Ambiguous cases were independently reviewed by 3 neuropathologists.

2.3. MGMT methylation analysis

MGMT promoter methylation status was determined using methylation-specific PCR (MSP) on extracted tumor DNA (*14*). Tumors were classified as MGMT-methylated if the methylated allele was detectable according to the assay protocol, and unmethylated otherwise. Of the 210 cases, 97 cases were evaluable for MGMT methylation, with the remaining cases lacking sufficient DNA for analysis.

2.4. Meta-analysis

The literature search strategy was designed around the

PICOT (*15*) question format: Do DMG patients (Patient problem) with the H3 K27-altered, including sex, age and anatomic location of tumor, (Intervention) compared to those under different conditions (Comparator), have a different prognosis of survival (Outcome) in observational cohort studies (Type of study)? Electronic searches were performed using Ovid Embase, PubMed, and Web of Science from their database inception dates to June 2024. A complete record of search strings is provided in Supplementary Text 1 (*https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*), and the PubMed search strategy is provided as an example. After excluding irrelevant studies, all data were extracted from article texts, tables, and figures with any estimates made based on the presented data and figures. Mean differences (MDs) in overall survival time and their corresponding 95% confidence intervals were extracted or estimated from the published text, tables, and figures and subsequently pooled in the meta-analysis. This was presented as a forest plot. The I^2 statistic was used to estimate the percentage of total variation across studies, owing to heterogeneity rather than chance, with values > 50% considered as substantial heterogeneity. Publication bias was assessed through the generation of funnel plots. All statistical analyses and bias in publication (Supplementary Figure S2, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*) were conducted with Review Manager (RevMan) version 5.2.

2.5. Statistical analysis

Survival data were evaluated utilizing Kaplan-Meier analysis in conjunction with the log-rank test. Differences between groups were assessed using the chi-square test and Fisher's exact test. Multivariable survival analysis was conducted employing the Cox proportional hazards model. For the Cox multivariable analysis, variables were selected based on their significance in univariable survival analyses. The proportional hazards assumption for all Cox regression models was assessed using Schoenfeld residuals tests. Both univariable and multivariable models were tested, with results reported as chi-square (χ^2) statistics with corresponding degrees of freedom (df) and *P*-values. A significance level of $P < 0.05$ was considered indicative of violation of the proportional hazards assumption (Supplementary Table S1, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*). Continuous variables, including tumor diameter and Ki-67 labeling index, were converted into categorical variables to enhance clinical interpretability and facilitate risk stratification. Specifically, a tumor diameter cutoff of 3 cm was applied, consistent with its common use in prior clinical and radiological studies (*16*) to pragmatically distinguish smaller from larger lesions. Similarly, a Ki-67 labeling index cutoff of 10% (*17*). Statistical analyses were executed using SPSS version 26.0 and R software. Heatmap was plotted by *https://www.bioinformatics.com.cn* (last accessed on 10 Dec 2024), an online platform for data analysis and visualization. A p-value of less than 0.05 was deemed to indicate statistical significance.

3. Results

3.1. Clinical characteristics

The clinical characteristics of 210 patients diagnosed with H3 K27M-altered DMG are detailed in Table 1. The mean age of the cohort was 26.44 years, with a median age of 26.0 years, spanning a range from 3 to 68 years. The primary tumor sites included the brainstem (86 out of 210 patients, 41.0%), thalamus (78 out of 210 patients, 37.1%), and spinal cord (25 out of 210 patients, 11.9%) (Figure 1, Figure 4A). The sex distribution comprised 102 males (48.6%) and 108 females (51.4%), while the age distribution included 122 adults (58.1%) and 88 children (41.9%). A total of 199 patients (94.8%) underwent surgical resection, whereas the remaining 11 patients (5.2%) received stereotactic biopsy. In adult patients, the thalamus was identified as the most prevalent tumor location, while in pediatric patients, the brainstem was the most frequently affected site. Sex-based stratification revealed that the thalamus was the predominant tumor location in male patients, whereas in female patients, the brainstem was most involved. The mean tumor diameter was calculated to be 4.49 cm (Figure 2).

Prior research has identified a higher incidence rate among children as a notable clinical feature of DMG. In order to further investigate the clinical characteristics within this case series, we stratified and summarized the cases according to sex and age. Statistical analyses demonstrated that tumors in adults were primarily located in the thalamus, whereas pediatric tumors were predominantly situated in the brainstem. Additionally, the incidence of DMG in the spinal cord was greater in adults compared to children (Table 2). From a sex-based perspective, tumors in females were predominantly located in the brainstem, whereas those in males were primarily concentrated in the thalamus (Table 3).

3.2. Molecular characteristics of H3 K27M- altered DMG

In most cases (129 out of 209, representing 61.7%), the Ki-67 proliferation index exceeded 10%. ATRX immunohistochemistry was conducted in 161 of the 210 patients, revealing that 115 cases (71.4%) were positive, and 46 cases (28.6%) were negative. Regarding p53 immunohistochemistry (n=208), 103 tumors (49.5%) tested positive, while 105 tumors (50.5%) tested negative. H3 K27M immunohistochemistry (n = 208) demonstrated that 198 tumors (95.2%) were positive,

Table 1. Clinical and Molecular Characteristics of 210 H3 K27M-mt DMGs

Character	Number
Age	
Adult (age above 18 y)	122 (58.1%, *n* = 210)
Pediatric (age 18 y or younger)	88 (41.9%, *n* = 210)
Sex	
Female	108 (51.4%, *n* = 210)
Male	102 (48.6%, *n* = 210)
Anatomic location	
Brainstem	86 (41.0%, *n* = 210)
Thalamus	78 (37.1%, *n* = 210)
Spinal cord	25 (11.9%, *n* = 210)
Other	21 (10.0%, *n* = 210)
Tumor diameter (cm)	
≤ 3	42 (20.5%, *n* = 205)
> 3	163 (79.5%, *n* = 205)
Molecular characteristics	
ATRX expression	
Positive	115 (71.4%, *n* = 161)
Negative	46 (28.6%, *n* = 161)
P53	
Positive	103 (49.5%, *n* = 208)
Negative	105 (50.5%, *n* = 208)
H3 K27M	
Positive	198 (95.2%, *n* = 208)
Negative	10 (4.8%, *n* = 208)
Ki-67(%)	
≤ 10	80 (38.3%, *n* = 209)
> 10	129 (61.7%, *n* = 209)
GFAP expression	
Positive	205 (98.0%, *n* = 209)
Negative	4 (2.0%, *n* = 209)
IDH1 expression	
Positive	7 (3.3%, *n* = 208)
Negative	201 (96.7%, *n* = 208)
OLIG2 expression	
Positive	198 (97.5%, *n* = 203)
Negative	5 (2.5%, *n* = 203)
MGMT promoter	
Methylated	7 (7.2%, *n* = 97)
Unmethylated	90 (92.8%, *n* = 97)
Median overall survival(months)	11

with 10 tumors (4.8%) being negative. Lastly, GFAP immunohistochemistry (n = 209) indicated that 205 tumors (98.0%) were positive, while 4 tumors (2.0%) were negative. In contrast, OLIG2 immunohistochemistry conducted on 203 tumors revealed that 198 tumors (97.5%) were positive, with only 5 tumors (2.5%) showing negative results (Figure 3, Figure 4A).

3.3. Survival data and prognostic factors

Comprehensive follow-up data were obtained for the entire cohort of 210 patients. During follow-up, 169 patients (80.5%) died. The median follow-up duration among surviving patients was 34 months. The median survival time was calculated to be 11.0 months, while the mean survival time was 16.5 months. The overall 1-year survival rate for the cohort was 46.2%, and the 2-year survival rate was 22.9%. The age-stratified analysis indicated that adults exhibited a more favorable prognosis

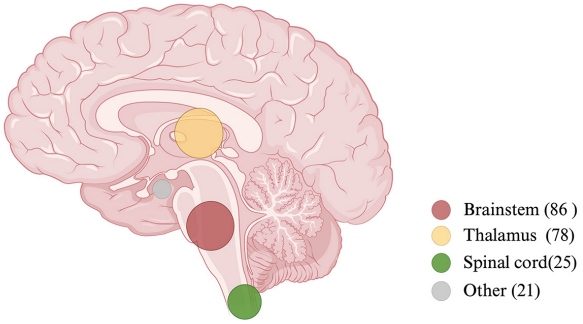


Figure 1. Anatomic and age distribution of H3 K27M-altered DMG.

than children (P = 0.006). No statistically significant difference in prognosis was observed between patients with tumor diameters greater than 3 cm and those with tumor diameters of 3 cm or less (P = 0.466). Analysis of tumor location demonstrated that individuals with brainstem tumors had a significantly poorer prognosis compared to those with tumors in other locations (P < 0.001). Furthermore, it was determined that males had a significantly better prognosis than females across the entire cohort (P = 0.014).

The data demonstrate that patients with p53-positive tumors exhibit a significantly poorer prognosis compared to those with p53-negative tumors (P = 0.003). Similarly, individuals with a Ki-67 index exceeding 10% have a worse prognosis than those with a lower Ki-67 index (P = 0.001). In contrast, no significant prognostic differences were identified based on ATRX status (positive or negative), H3 K27M status (positive or negative), OLIG2 status (positive or negative), GFAP status (positive or negative), or MGMT methylation status (methylated or unmethylated) (Figure 4B, Figure 5). Cases with missing data have been excluded from the analysis, and the missing ATRX and MGMT status data do not affect the conclusions (Supplementary Table S1, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*). In addition, we analyzed the associations between age and sex and prognosis in patients with different tumor locations; however, no statistically significant results were observed (Supplementary Figure S3, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*).

Schoenfeld residuals tests supported the proportional hazards assumption for all Cox regression models. No significant violations were detected in univariable analyses (all P > 0.05), and the multivariable model global test was non-significant (χ^2 = 11.78, df = 7, P = 0.11) (Supplementary Table S2, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*). In the multivariable Cox regression model, male sex (P = 0.023), spinal cord tumor location (P = 0.003), and a Ki-67 index of ≤ 10% (P = 0.039) were independently associated with favorable survival,

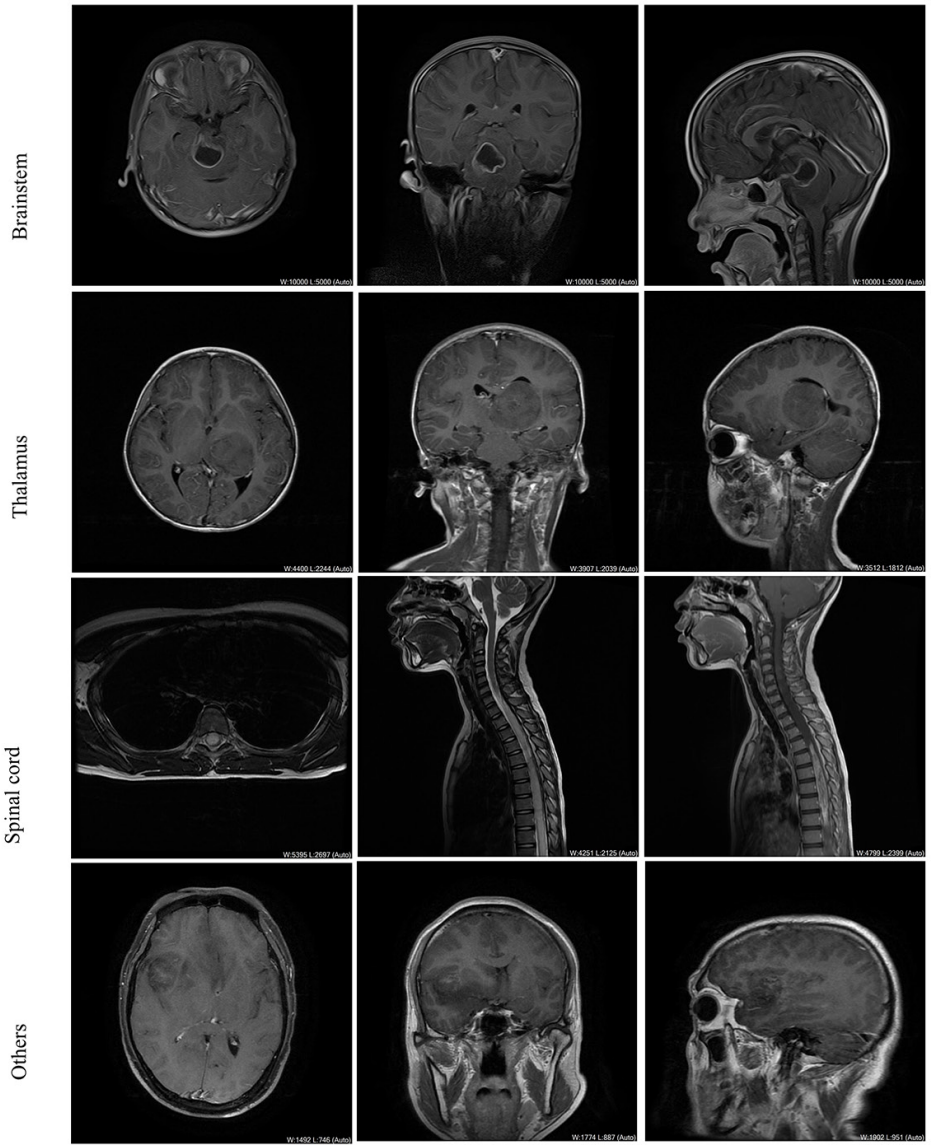


Figure 2. Representative imaging features of H3 K27M-altered DMG at different anatomical locations.

Table 2. Comparison of Clinical Characteristics Between Pediatric and Adult H3 K27M-altered DMG

	Adult (age above 18 y)	Pediatric (age 18 y or younger)	*P*-value
Case	122 (58.1%)	88 (41.9%)	0.069
Sex			
Female	56 (46.0%)	52 (59.1%)	
Male	66 (54.0%)	36 (40.9%)	
Anatomic location			< 0.001
Brainstem	32 (26.2%)	54 (61.4%)	0.014
Thalamus	54 (44.3%)	24 (27.3%)	0.029
Spinal cord	20 (16.4%)	5 (5.7%)	0.102
Other	16 (13.1%)	5 (5.7%)	0.157
Tumor diameter (cm)			
≤ 3	28 (23.7%)	13 (14.9%)	
> 3	90 (76.3%)	74 (85.1%)	

Table 3. Comparison of Clinical Characteristics Between Male and Female H3 K27M-altered DMG

	Female	Male	*P*-value
Case	108 (51.4%)	102 (48.6%)	
Age			0.069
Adult	56 (51.9%)	36 (35.3%)	
Pediatric	52 (48.1%)	66 (64.7%)	
Anatomic location			
Brainstem	54 (50.0%)	32 (31.4%)	0.008
Thalamus	33 (30.6%)	45 (44.1%)	0.046
Spinal cord	13 (12.0%)	12 (11.8%)	1.000
Other	8 (7.4%)	13 (12.7%)	0.251
Tumor diameter (cm)			0.862
≤ 3	22 (21.0%)	19 (19.0%)	
> 3	83 (79.0%)	81 (81.0%)	

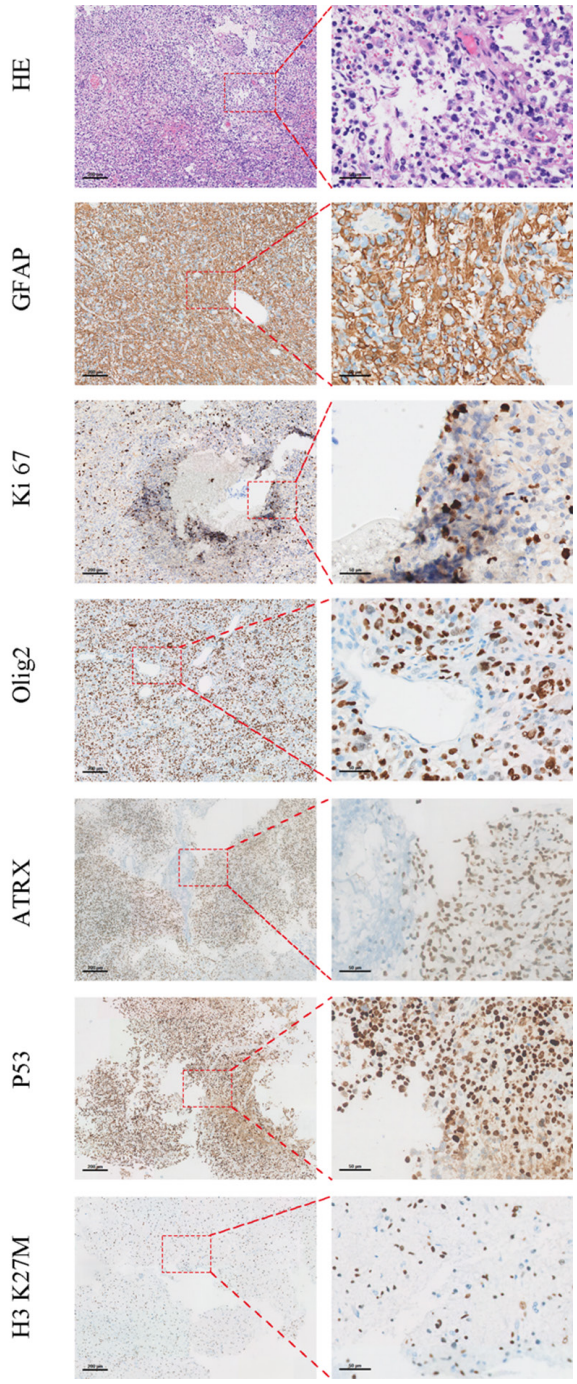


Figure 3. Representative hematoxylin and eosin (H&E) and immunohistochemical staining for GFAP, Ki-67, OLIG2, ATRX, p53, and H3 K27M.

whereas age and p53 status were not independently significant (Figure 6). To provide an exploratory visualization of the combined prognostic effects of the variables included in the multivariable Cox model, we constructed an overall survival (OS) nomogram. A higher total score, calculated as the sum of the scores assigned to each factor, corresponds to lower estimated 1-, 3-, and 5-year OS probabilities (Supplementary Figure S4, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*). Because the predictive performance of this nomogram has not been independently validated, it should be regarded as a preliminary prognostic tool rather than a model ready for routine clinical use.

3.4. Meta-analysis

Although this study constructed the largest known cohort of DMG to date to explore its clinical prognostic factors, the single-center nature of the research may introduce potential bias. To assess the consistency of our cohort findings with previously published evidence, we performed a supportive meta-analysis of relevant studies. A comprehensive retrieval process yielded a total of 8,180 articles. Following a rigorous screening procedure, 21 articles were selected for inclusion in the meta-analysis (Supplementary Figure S1, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*). The findings indicated that an age-stratified analysis demonstrated a significantly better prognosis for adults compared to children ($P = 0.002$, $I^2 = 47\%$). Furthermore, an analysis of tumor location revealed that patients with brainstem tumors exhibited a poorer prognosis relative to those with tumors situated in other locations ($P = 0.003$, $I^2 = 0\%$). No significant prognostic difference was observed between male and female patients ($P = 0.22$, $I^2 = 33\%$). The results of the analysis were illustrated using a forest plot (An I^2 value of less than 50% was considered indicative of acceptable heterogeneity, in which case the fixed effects model was employed; otherwise, the random effects model was adopted), and potential publication bias was evaluated through the construction of a funnel plot (Figure 7). Overall, the meta-analysis supported age and tumor location, but not sex, as comparatively reproducible prognostic factors across published cohorts.

4. Discussion

Diffuse midline gliomas constitute a heterogeneous category of tumors originating from the midbrain, pons, medulla, spinal cord, and thalamus and affect both pediatric and adult populations. These tumors exhibit diverse histological characteristics (*18*) and varying degrees of resectability, leading to heterogeneous clinical trajectories. The present study extends the current understanding of this heterogeneity by integrating clinicopathological variables, immunohistochemical markers, and survival outcomes in a large real-world cohort spanning both pediatric and adult patients. Rather than suggesting that prognosis is determined by any single factor, our findings indicate that survival may reflect the combined influence of patient characteristics, anatomical location, and tumor proliferative activity. Notably, the concordant associations of age and tumor location observed in both our cohort and the meta-analysis suggest that these variables may represent

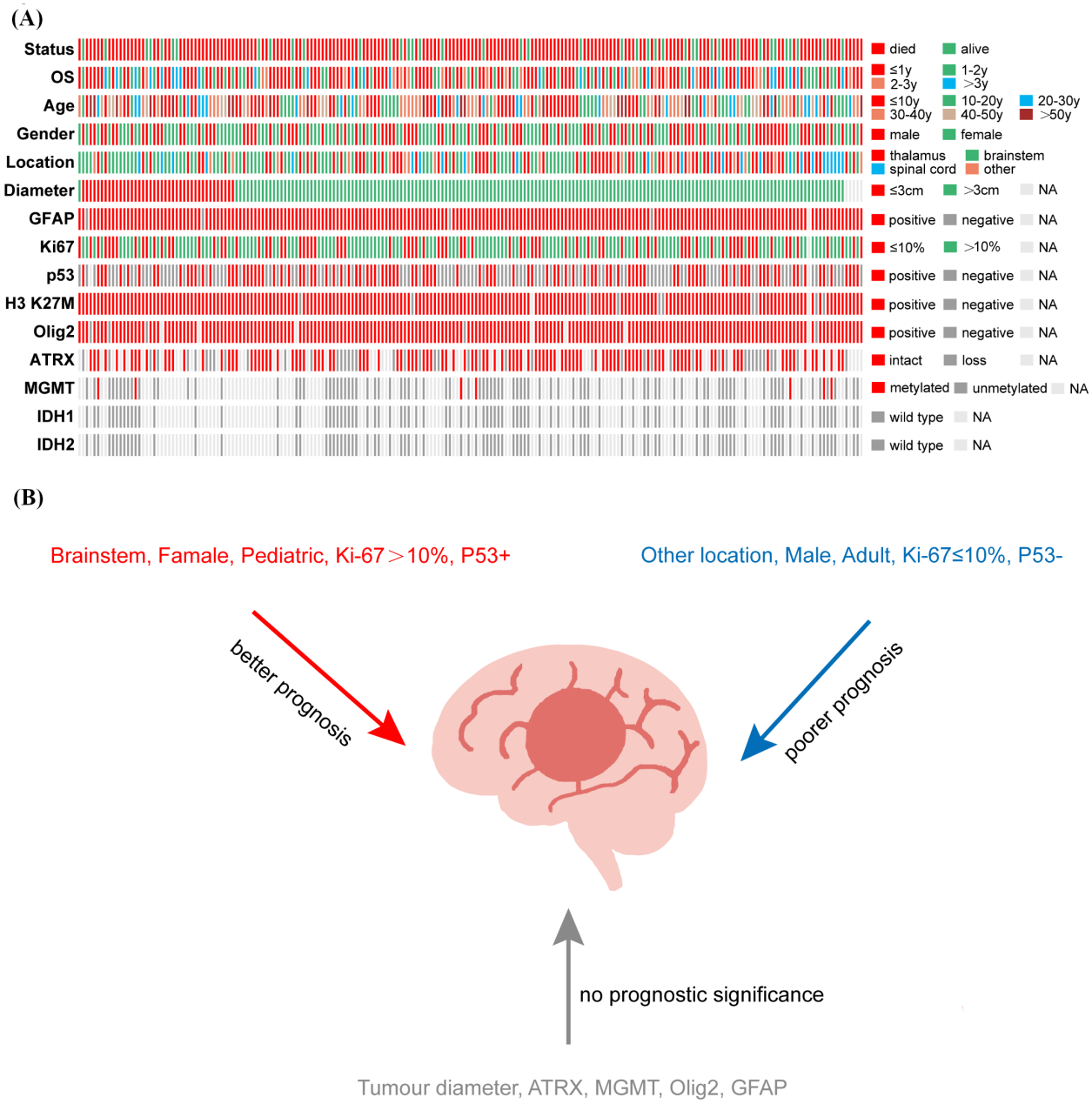


Figure 4. (A) Schematic summary of the clinical and pathological characteristics of 210 H3 K27M-altered DMG. (B) Impact of different variables on prognosis.

relatively robust prognostic indicators across different populations. In contrast, the sex-related association identified in the single-center cohort requires cautious interpretation and independent validation. These observations support a multidimensional approach to prognostic assessment and provide a rationale for incorporating routinely available clinicopathological variables into future risk-stratification frameworks.

Pediatric DMG has been closely associated with diffuse intrinsic pontine glioma, whereas advances in molecular classification have broadened the recognition of H3 K27-altered DMG across a wider age range and anatomical spectrum. Consistent with previous reports (*16*), tumors in adult patients in our cohort were more frequently located in the thalamus or spinal cord, whereas pediatric tumors were predominantly located in the brainstem. The more favorable survival observed in adults may therefore partly reflect differences in anatomical distribution, particularly the higher proportion of brainstem tumors among pediatric patients. However, because the location-stratified survival analyses did not reach statistical significance, the extent to which tumor location accounts for the age-related survival difference remains uncertain. Other age-associated biological or molecular differences may also contribute to the distinct clinical outcomes and warrant further investigation. These findings support the joint consideration of age and tumor location in prognostic assessment, while avoiding attribution of the survival difference to anatomical distribution alone.

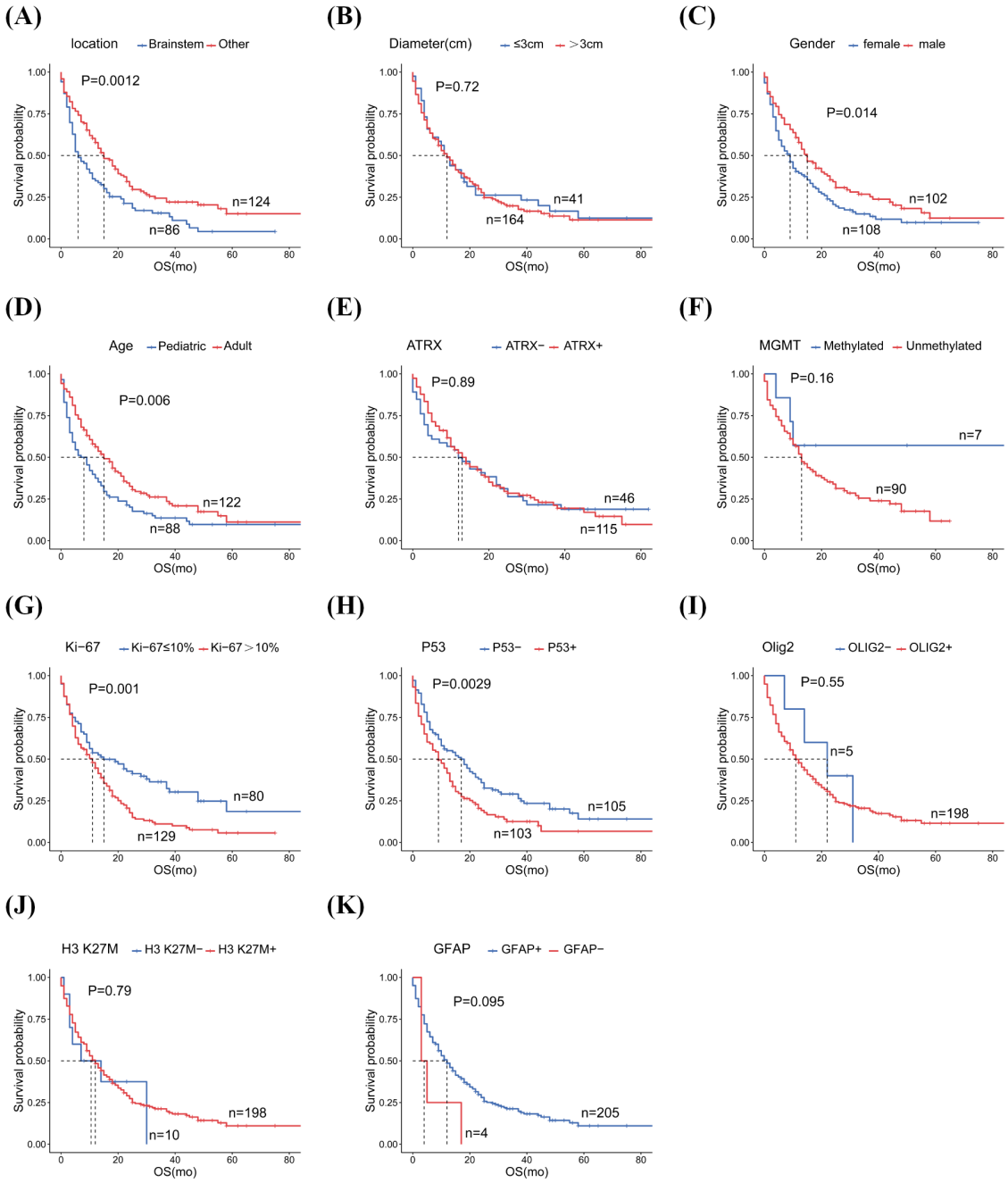


Figure 5. Kaplan–Meier curves for overall survival according to clinicopathological factors. Brainstem location **(A)**, female sex **(C)**, age ≤ 18 years **(D)**, a Ki-67 index > 10% **(G)**, and p53 positivity **(H)** were associated with poorer overall survival. Tumor diameter **(B)**, ATRX status **(E)**, MGMT promoter methylation status **(F)**, OLIG2 status **(I)**, H3 K27M status **(J)**, and GFAP status **(K)** were not significantly associated with overall survival.

Previous research did not recognize sex as a prognostic factor (*19*). However, our study found that males exhibited better overall survival than females. The observed survival advantage in males may be multifactorial. Male patients in our cohort more frequently presented with thalamic tumors, whereas females had a higher proportion of brainstem tumors, which are associated with poorer outcomes. This spatial distribution could partly explain the sex disparity in prognosis, although no significant association between sex and prognosis was observed among patients with tumors at different locations in our cohort (Supplementary Figure S3, *https://www.biosciencetrends.com/action/getSupplementalData.php?ID=311*). Prior studies have indicated a poorer prognosis for female pediatric patients compared to their male counterparts (*20*), and in our study, this observation extends across the entire age spectrum. Regarding sex differences in the incidence of gliomas and other cancers, extensive literature has documented these patterns (*21-23*), with some differences even linked to germline genetic variations (*24*). Previous studies in mouse models of glioma driven by Trp53 and

(A)

Variable	HR(95% CI)	*P* value
Age		
>18y	Reference	
≤18y	1.53 (1.13 to 2.07)	**0.006**
Gender		
Female	Reference	
Male	0.69 (0.51 to 0.93)	**0.015**
Tumor location		
Brainstem	Reference	
Thalamus	0.72 (0.52 to 1.01)	0.056
Spinal cord	0.32 (0.18 to 0.58)	**<0.001**
Other	0.66 (0.39 to 1.13)	0.129
Diameter		
>3cm	Reference	
≤3cm	0.93 (0.64 to 1.37)	0.727
ATRX expression		
Loss	Reference	
Intact	0.97 (0.66 to 1.44)	0.884
Ki67		
>10%	Reference	
≤10%	0.58 (0.42 to 0.81)	**0.001**
p53 positivity		
Negative	Reference	
Positive	1.59 (1.17 to 2.16)	**0.003**



(B)

Variable	HR(95% CI)	*P* value
Age		
>18y	Reference	
≤18y	1.14 (0.82 to 1.59)	0.442
Gender		
Female	Reference	
Male	0.69 (0.51 to 0.95)	**0.023**
Tumor location		
Brainstem	Reference	
Thalamus	0.81 (0.57 to 1.16)	0.251
Spinal cord	0.40 (0.22 to 0.74)	**0.003**
Other	0.75 (0.44 to 1.29)	0.299
Ki67		
>10%	Reference	
≤10%	0.70 (0.49 to 0.98)	**0.039**
p53 positivity		
Negative	Reference	
Positive	1.32 (0.95 to 1.83)	0.097

Figure 6. Univariable and multivariable Cox regression analyses of clinicopathological factors in H3 K27M-altered DMG. (A) Univariable analysis. **(B)** Multivariable analysis. Bold *P* values indicate statistical significance ($P < 0.05$).

Nf1 gene deletions reported longer survival in female mice, reflecting a sex-dimorphic survival pattern (*25*). In our study, sex as an independent prognostic factor may therefore have meaningful implications, though further validation in larger prospective cohorts is warranted.

In addition to demographic and anatomical factors, we evaluated the prognostic relevance of routinely available pathological markers. Among the pathological markers evaluated, Ki-67 provided the most consistent prognostic signal. This association is biologically plausible because a higher Ki-67 index reflects increased proliferative activity and may identify tumors with more aggressive growth (*16,26*). In contrast, p53 immunopositivity was associated with survival in univariable analysis but did not retain independent significance after adjustment. Because p53 staining is an indirect surrogate of TP53 pathway disturbance rather than a direct genomic measurement, its prognostic interpretation should remain cautious. ATRX, GFAP, and OLIG2 were not associated with survival, suggesting that markers useful for diagnostic or lineage characterization do not necessarily provide independent prognostic information.

Furthermore, the prognostic value of MGMT promoter methylation in H3 K27M-altered DMG remains inconclusive in the present study. Notably, MGMT promoter methylation was detected in only a small proportion of cases (7.2%), which substantially limits the statistical power to identify a potential survival difference between methylated and unmethylated tumors. Therefore, the absence of a significant association between MGMT methylation status and overall survival in our cohort should be interpreted with caution and should not be considered definitive evidence of a lack of prognostic relevance. Given the low prevalence of MGMT methylation in DMG and the limited sample size of methylated cases, larger multicenter cohorts or pooled molecular datasets will be required to more reliably evaluate its prognostic and therapeutic implications.

Currently, the primary treatment modalities for DMG include surgical resection and a combination of radiotherapy and chemotherapy (*27*). While surgical resection and focal radiotherapy can temporarily alleviate symptoms associated with DMG, they do not significantly extend patient survival (*28*). Nevertheless, a multicenter retrospective study conducted at Bellaria Hospital in Bologna, Italy, indicated that the concurrent administration of radiotherapy and chemotherapy following surgery is associated with improved patient survival outcomes (*29*). A subset of recently identified targeted therapies or modified drug regimens demonstrates potential therapeutic efficacy (*30*). Although immunotherapy has shown promise in treating non-central nervous system solid tumors, its effectiveness in intracranial tumors remains limited (*31*). To date, only therapies such as CAR-T-cell therapy (*32-34*) and virotherapy (*35,36*) have yielded promising outcomes in preliminary clinical trials. However, to substantiate these findings, further multicenter clinical trials are necessary, necessitating additional research to identify effective pharmacological interventions.

From a clinical perspective, age, tumor location, Ki-67 index, and p53 status are readily available variables that may complement molecular classification and clinical judgment when estimating prognosis; sex may be retained as an exploratory factor pending validation. Their combined use may support patient counseling, follow-up planning, and more balanced stratification in future biomarker-driven trials. Compared with

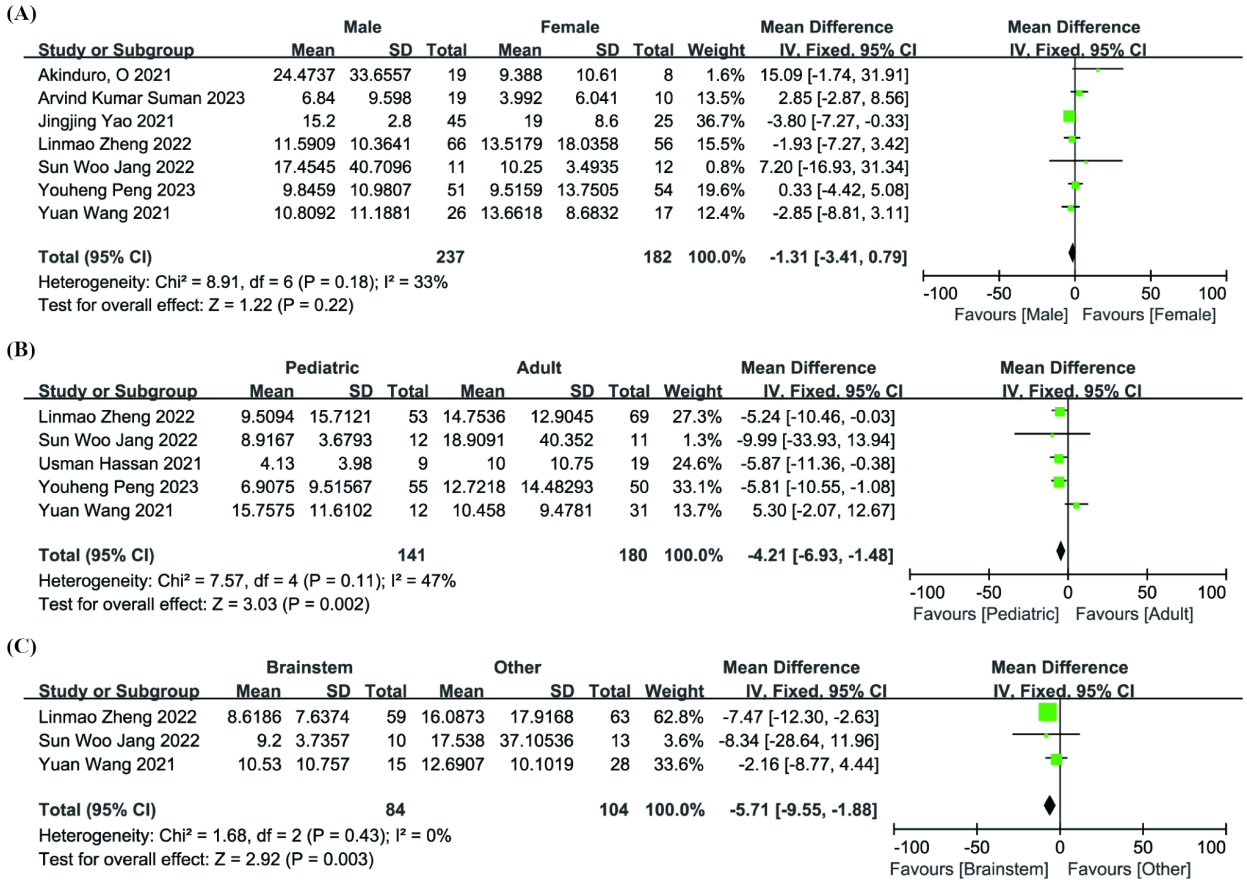


Figure 7. Forest plots of pooled mean differences in mean overall survival time according to sex (A), age group (B), and tumor location (C) in patients with H3 K27M-altered DMG.

previous reports (*16,19*), the major contribution of the present work lies not only in the relatively large cohort but also in the integration of clinicopathological characteristics, molecular marker evaluation, and long-term survival analyses within a real-world clinical setting. This comprehensive approach provides a more complete characterization of H3 K27-altered DMG across both pediatric and adult populations and offers clinically relevant evidence for prognostic assessment and risk stratification. Furthermore, the identified clinicopathological prognostic factors may facilitate risk-adapted patient management, support individualized clinical decision-making, and provide a practical foundation for future biomarker-driven clinical trials.

Nonetheless, our study is limited by its single-center retrospective design, the predominance of surgically resected tumors, limited statistical power for subgroup analyses, and the absence of more comprehensive genomic and epigenomic data, such as ACVR1 or PDGFRA mutations and histone modification profiles. Because next-generation sequencing and DNA methylation profiling were not available, the molecular characterization remained primarily limited to immunohistochemical markers. Consequently, we were unable to distinguish H3 molecular subtypes or comprehensively assess key co-occurring alterations such as ACVR1, FGFR1, TERT, and PDGFRA. Future studies incorporating comprehensive molecular profiling are warranted to address these limitations. In addition, detailed treatment-related variables, including extent of resection (biopsy/STR/GTR), postoperative radiotherapy, and chemotherapy, were not systematically available and therefore could not be incorporated into the multivariable Cox model. Consequently, residual confounding related to treatment modalities cannot be completely excluded and may have influenced the observed survival outcomes. The nomogram was also developed from the same single-center cohort, and its discrimination, calibration, and generalizability have not yet been fully evaluated. Prospective validation and independent external validation are therefore required before the model can be considered for routine clinical implementation. Furthermore, the meta-analysis was intended primarily as supportive validation of the single-center cohort rather than as an independent confirmatory analysis. Because it was conducted in a simplified manner and did not include formal quality assessment or detailed study-level summary tables, its findings should be interpreted as supportive rather than definitive evidence. This work is primarily based on clinical and statistical analyses, and no mechanistic experiments were performed to elucidate the underlying biological

pathways. Future multicenter studies integrating molecular, genomic, and therapeutic data are warranted to validate and expand upon these findings.

5. Conclusion

This study advances the current understanding of H3 K27-altered diffuse midline glioma by providing an integrated clinicopathological and prognostic evaluation of a large real-world cohort spanning pediatric and adult patients. Our findings highlight the combined prognostic relevance of patient age, tumor location, sex, p53 expression, and proliferative activity, while indicating that the strength and independence of these associations differ across variables. In particular, the concordant findings from the single-center cohort and meta-analysis support age and tumor location as comparatively reproducible prognostic factors, whereas the sex-related association should remain exploratory pending further validation. The identified clinicopathological factors are routinely available and may contribute to future clinical risk stratification, patient counseling, follow-up planning, and the design of more balanced biomarker-driven clinical trials. However, their clinical implementation requires validation in independent multicenter and prospective cohorts. Future studies integrating comprehensive genomic, epigenomic, and treatment-related data will be essential to refine prognostic models, clarify the biological basis of clinical heterogeneity, and support the development and evaluation of risk-adapted therapeutic strategies for H3 K27-altered DMG.

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Data Availability statement: The data generated in this study are available within the article and its supplementary data files.

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