Received: 5 August 2023 | Accepted: 18 December 2023

DOI: 10.1111/bpa.13233

RESEARCH ARTICLE

Brain Pathology

Refinement of prognostication for *IDH*-mutant astrocytomas using DNA methylation-based classification

Teresia Kling[1] | **Sandra Ferreyra Vega**[1] | **Medha Suman**[1] | **Anna Dénes**[2] | **Anna Lipatnikova**[2] | **Stina Lagerström**[1] | **Thomas Olsson Bontell**[3,4] | **Asgeir Store Jakola**[2,5] | **Helena Carén**[1]

[1]Sahlgrenska Center for Cancer Research, Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[2]Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[3]Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[4]Department of Clinical Pathology, Sahlgrenska University Hospital, Gothenburg, Sweden

[5]Department of Neurosurgery, Sahlgrenska University Hospital, Gothenburg, Sweden

Correspondence
Helena Carén, Sahlgrenska Center for Cancer Research, Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Medicinaregatan 1F, 405 30 Gothenburg, Sweden.
Email: helena.caren@gu.se

Funding information
The Swedish state under the agreement between the Swedish government and the county councils - the ALF-agreement, Grant/Award Numbers: ALFGBG-965622, ALFGBG-965828; Vetenskapsrådet; Cancerfonden; Swedish Research Council

Abstract

The 2021 World Health Organization (WHO) grading system of isocitrate dehydrogenase (*IDH*)-mutant astrocytomas relies on histological features and the presence of homozygous deletion of the cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2A*/*B*). DNA methylation profiling has become highly relevant in the diagnosis of central nervous system (CNS) tumors including gliomas, and it has been incorporated into routine clinical diagnostics in some countries. In this study, we, therefore, examined the value of DNA methylation-based classification for prognostication of patients with *IDH*-mutant astrocytomas. We analyzed histopathological diagnoses, genome-wide DNA methylation array data, and chromosomal copy number alteration profiles from a cohort of 385 adult-type *IDH*-mutant astrocytomas, including a local cohort of 127 cases and 258 cases from public repositories. Prognosis based on WHO 2021 CNS criteria (histological grade and *CDKN2A*/*B* homozygous deletion status), other relevant chromosomal/gene alterations in *IDH*-mutant astrocytomas and DNA methylation-based subclassification according to the molecular neuropathology classifier were assessed. We demonstrate that DNA methylation-based classification of *IDH*-mutant astrocytomas can be used to predict outcome of the patients equally well as WHO 2021 CNS criteria. In addition, methylation-based subclassification enabled the identification of *IDH*-mutant astrocytoma patients with poor survival among patients with grade 3 tumors and patients with grade 4 tumors with a more favorable outcome. In conclusion, DNA methylation-based subclassification adds prognostic information for *IDH*-mutant astrocytomas that can further refine the current WHO 2021 grading scheme for these patients.

KEYWORDS
CDKN2A/*B* homozygous deletion, diagnosis, DNA methylation profiling, *IDH*-mutant astrocytomas, prognosis, WHO 2021 CNS criteria

Teresia Kling and Sandra Ferreyra Vega contributed equally to this study and are co-first authors.

1 | INTRODUCTION

Adult-type diffuse isocitrate dehydrogenase (*IDH*)-mutant gliomas are malignant tumors of the central nervous system (CNS) that are characterized by somatic mutations in the *IDH* genes 1 and 2. The tumors are highly infiltrative and display variable clinical and biological behavior and the prognosis for the patients remains relatively poor [1]. According to the current World Health Organization (WHO) 2021 classification of CNS tumors *IDH*-mutant astrocytomas are graded based on histomorphological criteria and cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2A*/*B*) homozygous deletion status [2]. The presence of *CDKN2A*/*B* homozygous deletion results in a CNS WHO grade 4, also in cases without morphological grade 4 criteria (e.g., microvascular proliferation and/or necrosis). The distinction between CNS WHO grades 2 and 3 is made solely based on histopathological examination. Focal amplification of *CDK4*, *MYCN*, and *PDGFRA* as well as burden of chromosomal aberrations have been proposed for prognostication of *IDH*-mutant astrocytomas [3]. Yet, there is insufficient evidence of their prognostic effect, and further evaluation is required.

DNA methylation is an epigenetic modification with a major effect on gene expression, and dysregulation of methylation patterns has been tightly linked to numerous diseases such as cancer [4, 5]. Mapping of genome-wide DNA methylation patterns has become highly relevant in the classification of CNS tumors as it has been shown to improve the diagnostic and prognostic accuracies [6–9]. Further, this technique has been incorporated into routine clinical diagnostics in many countries [8, 10–12] and more will likely follow, given the newly updated WHO 2021 CNS recommendations. According to the most commonly used brain tumor methylation classifier, *IDH*-mutant astrocytomas can display methylation patterns associated with the "lower grade" or the "high grade" methylation subclasses [13]. The concordance of this methylation-based risk-stratification with the WHO 2021 CNS grading scheme is unknown, yet it could potentially complement and refine the current grading and prognostication of these tumors as well as to provide guidance for treatment decision-making.

In this study, we, therefore, examine the added prognostic value of chromosomal/gene alterations and DNA methylation-based subclassification in patients with *IDH*-mutant astrocytomas in relation to the current WHO 2021 CNS grading scheme.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Our local cohort consisted of patients ≥17 years old with primary *IDH*-mutant lower grade astrocytomas (WHO 2016 grades II and III, $n = 107$) or histological grade IV *IDH*-mutant glioblastomas (WHO 2016, $n = 20$) diagnosed between 2007 and 2022 at the Department of Neurosurgery, Sahlgrenska University Hospital (Gothenburg, Sweden). Formalin-fixed paraffin-embedded (FFPE) tumor tissue and corresponding clinical patient data from cases between 2007 and 2016 were collected and processed in a previous study [7]. FFPE samples and corresponding clinical patient data from 2017 to 2022 were collected retrospectively for this study at the Department of Pathology, Sahlgrenska University Hospital. We used fresh-frozen (FF) tumor tissue samples from cases with unavailable FFPE samples for methylation analyses ($n = 4$). Mutation status of *IDH1* or *IDH2* and retention of 1p and 19q chromosomal arms were confirmed at the time of diagnosis or retrospectively assessed as previously described [7]. For patient data, see Tables S1–S3.

2.2 | DNA methylation profiling

DNA was isolated from FFPE tumor samples with the Maxwell® FFPE Plus DNA Kit using the Maxwell® RSC Instrument (Promega, Madison, Wisconsin, USA) as indicated by the developers. Extraction of DNA from FF tumor samples was carried out with the DNeasy Blood and Tissue Kit and TissueLyser (Qiagen, Hilden, Germany) following the manufacturer's instructions. A total of 500 ng DNA from both FFPE and FF tumors was bisulfite modified (BSM) with the EZ DNA methylation kit (Zymo Research, Irvine, California, USA). BSM DNA from FFPE samples was further restored with Infinium HD FFPE DNA Restore Kit (Illumina, San Diego, California, USA). All samples were then processed on the Infinium Methylation EPIC (850 k) array (Illumina) according to protocols supplied by the manufacturer.

2.3 | Methylation data analysis

The molecular neuropathology (MNP) brain classifier version 12.5 (unpublished, https://www.molecularneuropathology.org/mnp) [13] was used for tumor classification into defined methylation class families (calibrated score ≥0.90) and subclasses (calibrated score ≥0.50). Raw methylation array data (idats) were processed using the statistical software R version 4.2.2 [14] with the R-package minfi [15]. We calculated the number of differentially methylated CpG positions (DMPs, $\Delta\beta \geq 0.20$, adjusted p-value <0.01) between the methylation subclasses "lower grade" and "high grade" *IDH*-mutant astrocytomas with ChAMP [16] and performed a Gene set enrichment analysis of these DMPs applying the GOmeth method as indicated [17]. Differentially methylated regions (DMRs, adjusted p-value <0.01) were identified with Probe Lasso in ChAMP [16].

2.4 | Chromosomal copy number alterations analysis

Chromosomal copy number alterations (CNAs) were retrieved from the methylation array data using the R-package conumee [18]. Focal gene amplifications (including *CDK4*, *MYCN*, and *PDGFRA*) were defined with a log2ratio value >0.40 [10, 19]. For *CDKN2A/B* copy number analysis, a log2ratio value of less than or equal to −0.40 at the *CDKN2A/B* locus was considered a homozygous deletion, and a log2ratio value greater than −0.4 was denominated "retained *CDKN2A/B*" [10, 19]. We further examined copy number burden by measuring the number of chromosomal breakpoints with abs(log2(value)) > 0.2.

2.5 | Public datasets

Bulk tumor DNA methylation array data (Infinium HumanMethylation450 BeadChip or EPIC 850 k) of *IDH*-mutant lower grade gliomas and glioblastomas were downloaded from The Cancer Genome Atlas (TCGA $n = 249$) [20, 21], CPTAC-3 project ($n = 6$) [22] and GSE60274 ($n = 3$) [23] repositories. Only *IDH*-mutant astrocytic glioma cases with retained 1p/19q chromosomal arms were processed as described above for our local cohort.

2.6 | Statistical analyzes

Overall survival was calculated by the Kaplan–Meier method (curves plotted with the R-package survminer [24]), measured from the date of diagnosis to the date of death or date of last follow-up (January 1, 2022). Survival curves were compared with the log-rank test using the R-package survival [25]. Hazard ratios (HRs) were determined using Cox proportional hazards multivariate regression and 95% confidence intervals (plotted with the R-package survminer [24]). We analyzed methylation subclasses and WHO 2021 CNS criteria as risk factors associated with patient survival. *p*-values of less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Description of study cohorts

A total of 127 patients with *IDH*-mutant astrocytic gliomas (WHO 2016) were included in the local cohort (Tables S1–S3). The patients' age at diagnosis ranged from 17 to 71 years, with a mean age of 39 years. Most of the tumors were histologically grade II (43%, $n = 54$) or grade III (42%, $n = 53$), whereas 16% ($n = 20$) of the cases were regarded as grade IV tumors, Figure 1A. Of the 127 cases, 9% ($n = 12$) harbored *CDKN2A/B* homozygous deletions as determined from CNA profiles retrieved from the methylation array data. Integration of histological grade and *CDKN2A/B* status of the 127 cases resulted in 22% WHO 2021 CNS grade 4, 35% grade 3, and 43% grade 2 tumors. The tumors were classified employing the MNP methylation classifier (v12.5), which classified the majority of the tumors as *IDH*-mutant diffuse gliomas (114 of 127) with calibrated scores >0.90. Among the 127 cases, 14% ($n = 18$) were subclassified as "high grade" and 76% ($n = 96$) as "lower grade" astrocytoma methylation subtypes (calibrated scores ≥0.50). The remaining cases were classified and/or subclassified with low calibrated scores (<0.9 or 0.30–0.50, respectively), which included cases subclassified as "high grade" or "lower grade" *IDH*-mutant astrocytomas ($n = 6$) or as other methylation classes than the *IDH*-mutant astrocytoma-glioma class ($n = 5$, e.g., ganglioglioma and control tissue, cerebral hemisphere). Two other cases were unclassified (calibrated scores <0.30) by the classifier tool (Table S1).

Similarly, among the 258 cases from the public datasets, 42% ($n = 108$) were histologically grade II *IDH*-mutant astrocytomas, followed by 39% ($n = 101$) grade III and 6% ($n = 16$) grade IV tumors. The remaining 13% ($n = 33$) of the cases had undetermined histological grade [20, 21], Figure 1B. Among the 258 cases, 14% ($n = 37$) harbored homozygous deletion of *CDKN2A/B*, leading to the assignation of 19% of the tumors as WHO 2021 CNS grade 4, 31% as grade 3 and 40% as grade 2. Methylation-based classification of the tumor cases resulted in 12% ($n = 31$) of the tumors subclassified as *IDH*-mutant astrocytomas of "high grade" and 86% ($n = 223$) were assigned to the "lower grade" subclass (calibrated score ≥0.50).

In both the local and public cohorts, the "lower grade" astrocytoma subclass consisted mainly of histological grade II and III tumors, whereas the grade IV tumors were more commonly subclassified as "high grade" astrocytomas, Figure 1C. *CDKN2A/B* homozygous deletions were predominantly found among the histological grade III tumors (local cohort [$n = 8$], public cohort [$n = 21$]) followed by grade IV tumors (local cohort [$n = 4$], public cohort [$n = 4$]). Among the histological grade II astrocytomas, six tumors of the public cohort showed homozygous deletion of *CDKN2A/B*, whereas this CNA was absent in the grade II tumors of the local cohort.

We further evaluated the frequency of relevant gene amplifications (*CDK4*, *MYCN*, and *PDGFRA*) as prognostic markers for *IDH*-mutant astrocytomas. Amplification of *CDK4* was mostly present in the histological grade III and IV tumors and in tumors subclassified with the "high grade" methylation subclass (local cohort [$n = 7$], public cohort [$n = 8$]), Figure S1. Only a few tumors in the local ($n = 2$) and public

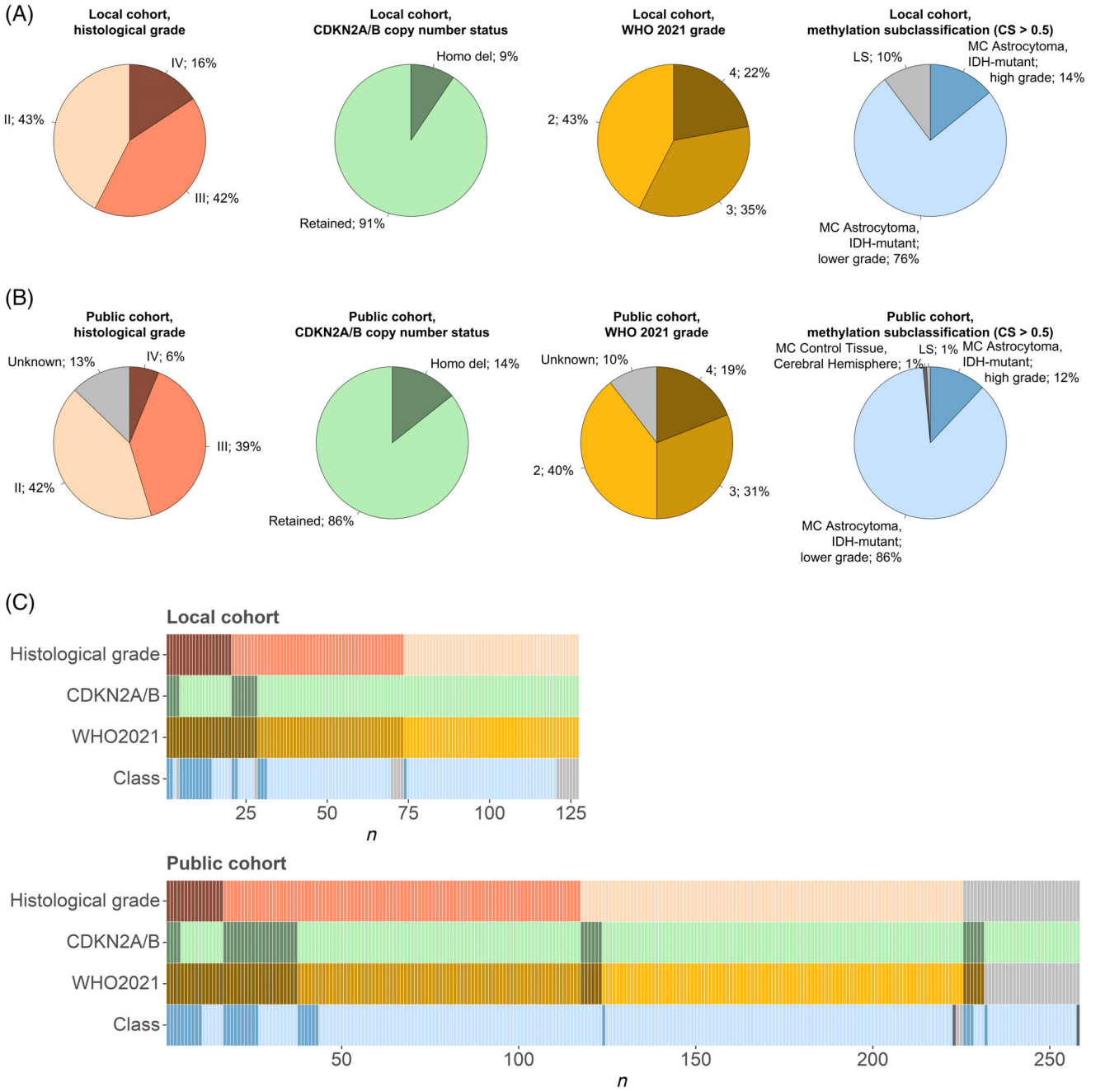

FIGURE 1 Prognostic grading of *IDH*-mutant astrocytomas in the (A) local cohort ($n = 127$) and (B) the public cohort ($n = 258$) based on histological grade, *CDKN2A*/*B* copy number status, WHO 2021 grade, and DNA methylation-based subclassification. (C) Prognostic grading per individual patient cases in the local cohort (top panel) and public cohort (bottom panel). The color scheme in (A and B) applies to (C). CS, calibrated score; Homo del, homozygous deletion; LS, low score (subclass CS <0.5); MC, methylation class.

cohorts ($n = 2$) showed *MYCN* amplifications and the great majority of these tumors were "high grade" based on their methylation profiles. *PDGFRA* amplifications were neither associated with a specific histological grade nor a methylation subclass. *CDK4*, *MYCN*, and *PDGFRA* amplifications were absent in the histological grade IV tumors subclassified as "lower grade" and histological grade II tumors.

3.2 | Prediction of overall survival by WHO 2021 CNS grading system and DNA methylation-based subclassification

We examined histological grade, *CDKN2A*/*B* homozygous deletion status and DNA methylation subclasses as independent prognostic markers for *IDH*-mutant astrocytomas in the local and public cohorts. Each of the three markers,

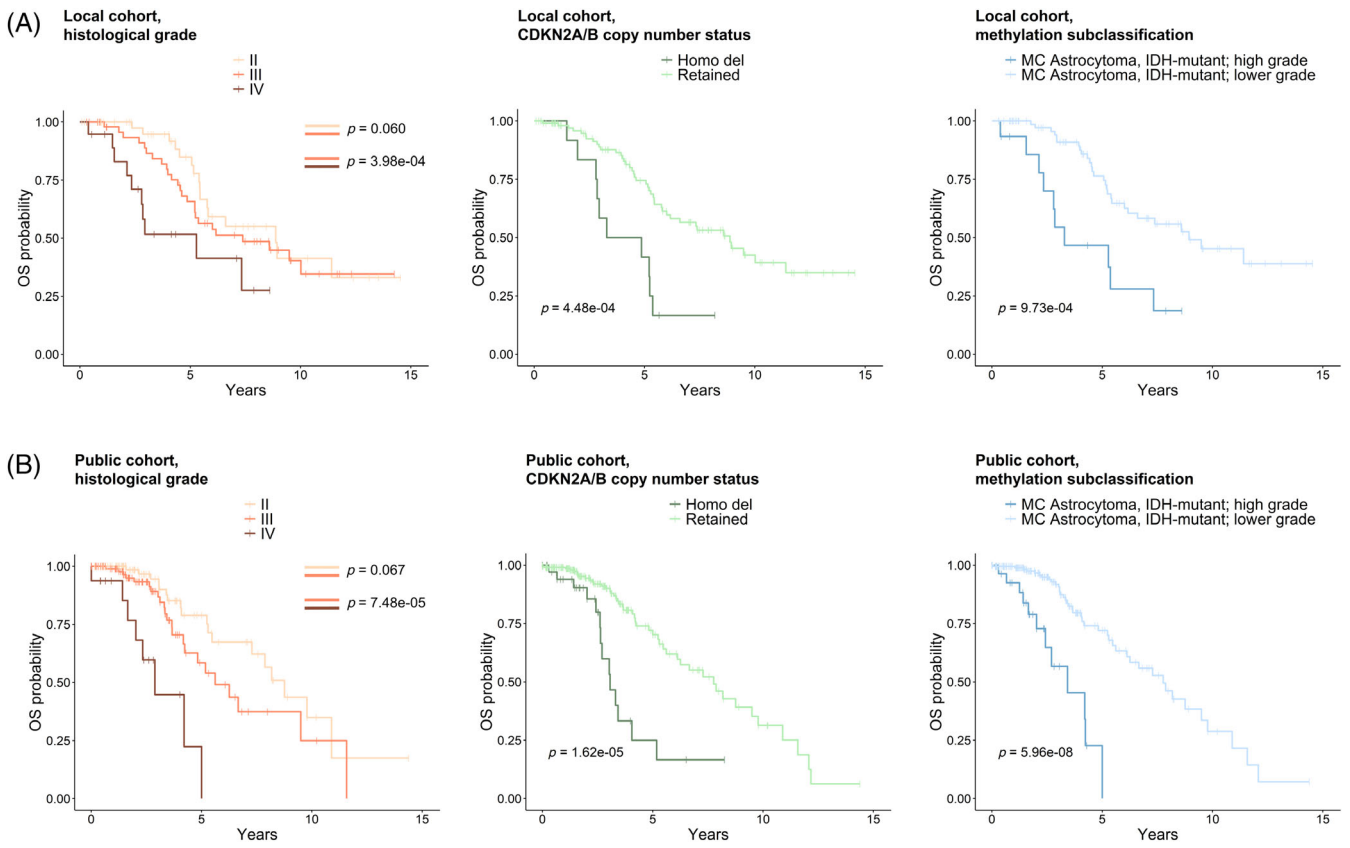

FIGURE 2 Overall survival (OS) probabilities of the patients in the (A) local cohort and (B) the public cohort stratified by left: histological grade, middle: *CDKN2A*/*B* copy number status, and right: DNA methylation-based subclassification. Significance: $p < 0.05$. Homo del, homozygous deletion; MC, methylation class.

histological grade IV, *CDKN2A*/*B* homozygous deletion or methylation-based subclassification as "high grade" astrocytoma, could individually separate the patient cases into prognostic groups with different overall survival ($p < 0.05$, Figure 2).

3.3 | DNA methylation-based subclassification refines prognostication for *IDH*-mutant astrocytomas

To better compare the risk prediction of the *IDH*-mutant astrocytoma methylation subclasses with WHO 2021 CNS grading criteria (i.e., histological grade and *CDKN2A*/*B* homozygous deletion status), we combined clinical and methylation array data from the local and public cohorts. Tumor cases subclassified with calibrated scores <0.50 by the MNP classifier [13] and TCGA cases with undetermined histological grade [20, 21] were excluded for additional analyses. In this final cohort of 336 tumors, we observed that the methylation-based subclassification ($p = 2.00e-09$) achieved a comparable risk stratification of patients as *CDKN2A*/*B* homozygous deletion status ($p = 3.78e-06$, Figure 3). The "high grade" *IDH*-mutant astrocytoma methylation subclass was further able to provide prediction of patient survival as well as the grade 4 WHO 2021 CNS criteria. We then compared the prognostication of *IDH*-mutant astrocytoma methylation subclasses with *CDK4*, *MYCN*, and *PDGFRA* amplifications and found that the "high grade" methylation subclass performed better than *CDK4* ($p = 3.57e-02$) and *MYCN* ($p = 1.83e-04$) amplifications in predicting survival of the patients, Figure S2. *PDGFRA* amplification was not associated with poor survival in our cohort.

We next examined whether an integrated analysis of methylation-based subclassification and the WHO 2021 CNS grading criteria could further improve the prognostic grading of *IDH*-mutant astrocytomas. Among the WHO 2021 CNS grade 4 *IDH*-mutant astrocytomas, 49% ($n = 34$) were assigned to the "high grade" methylation subclass, whereas 7% ($n = 9$) grade 3 and 1% ($n = 2$) grade 2 cases fall into this methylation subclass (Figure 4A). We observed that the "high grade" methylation subclass provided a refined prognostication for patients with WHO 2021 CNS grade 3 tumors as this subclass identified patients with worse survival than the "lower grade" methylation subclass ($p = 0.019$, Figure 4B). Similarly, the "lower grade" methylation subclass identified an intermediate prognostic subgroup for

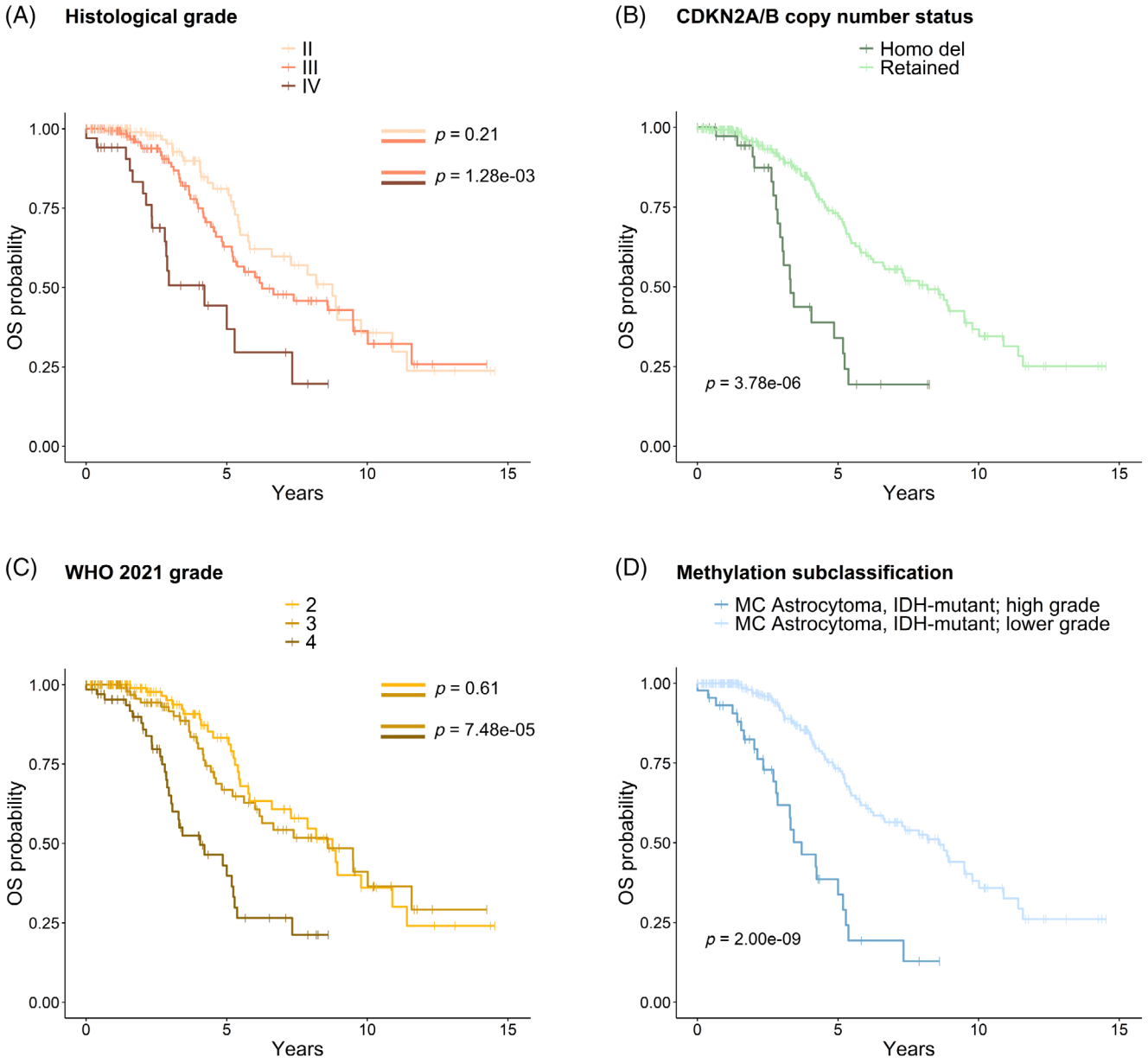

FIGURE 3 Overall survival (OS) probabilities of 336 patients with *IDH*-mutant astrocytomas in the final combined cohort stratified by (A) histological grade, (B) *CDKN2A/B* copy number status, (C) WHO 2021 CNS grading criteria and (D) DNA methylation-based subclassification. Significance: $p < 0.05$. Homo del, homozygous deletion; MC, methylation class.

patients with WHO 2021 CNS grade 4 tumors, as these patients clearly showed a prolonged survival outcome compared with patients with the "high grade" subclass ($p = 0.038$, Figure 4B). Thus, in 23% of cases with WHO 2021 CNS grade 3 and grade 4 tumors, the methylation subclass may refine the prognostication.

Cox multivariate regression model revealed that *CDKN2A/B* homozygous deletion status (HR: 2.6, p <0.001) and methylation-based subclassification (HR: 2.2, $p = 0.019$) were independent prognostic factors for *IDH*-mutant astrocytomas but not histological grade (Figure S3). Methylation-based subclassification (HR: 2.3, $p = 0.01$) remained an independent prognostic factor when compared with WHO 2021 CNS grading criteria (HR: 2.1, $p = 0.024$) for these patients, Figure 4C.

3.4 | Molecular characterization of the *IDH*-mutant astrocytoma methylation subclasses

Because the *IDH*-mutant astrocytoma methylation subclasses can be used for prognostication of the tumors, we sought to determine the potential mechanisms characterizing the "high grade" and "lower grade" methylation subclasses. We first characterized the number of chromosomal

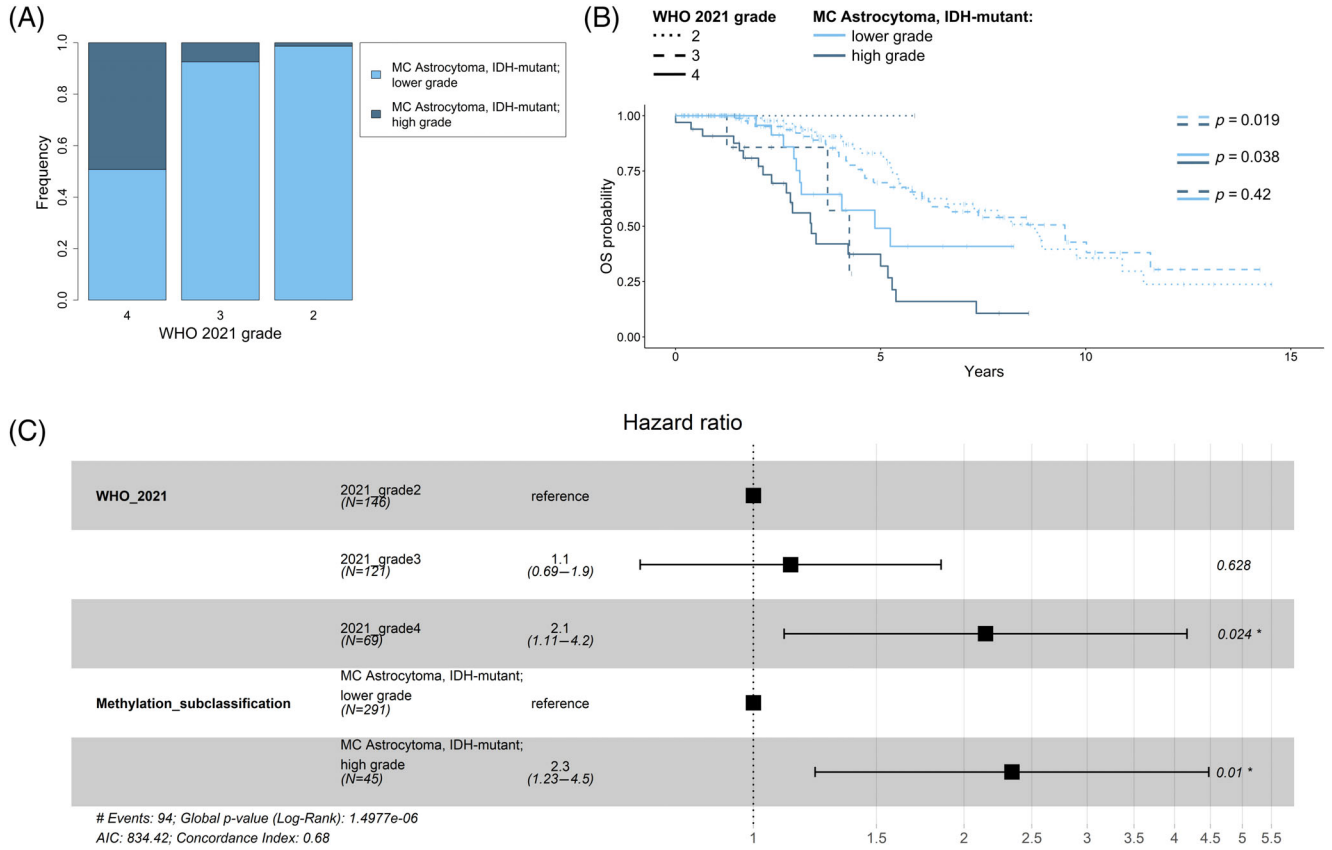

FIGURE 4 Integrated prognostication of *IDH*-mutant astrocytomas according to WHO 2021 CNS criteria and DNA methylation-based subclassification. (A) Frequency of WHO 2021 grade 2–4 *IDH*-mutant astrocytoma cases stratified by "high grade" and "lower grade" methylation subclasses. (B) Incorporation of methylation subclasses to the WHO 2021 CNS grading criteria refined prognostication of grade 3 and grade 4 patients. Significance: $p < 0.05$. (C) Cox proportional regression model of overall survival based on top: WHO 2021 CNS grades (grade 2, 3, and 4), and bottom: *IDH*-mutant astrocytoma methylation subclasses ("lower grade" and "high grade"). Methylation-based subclassification had a risk association comparable to WHO 2021 CNS criteria. (*) denotes significance ($p < 0.05$). Homo del, homozygous deletion; MC, methylation class.

breakpoints, as an increased CNA burden has been associated with poor clinical behavior in patients with *IDH*-mutant astrocytomas [19, 26–28]. Our results revealed significantly increased levels of chromosomal breakpoints in the "high grade" methylation subclass compared with the "lower grade" methylation subclass ($p = 8.01e\text{-}10$, Figure 5A). We further performed differential methylation analysis and found a total of 19,829 significant DMPs (adjusted *p*-value <0.01) occurring between the "high grade" and "lower grade" methylation subclasses (Table S4). Enrichment analysis of these DMPs identified biological pathways related to sensory perception/detection of stimulus, and distinct receptor activity functions (Figure 5B). Differential analysis of genomic regions identified 151 significant DMRs (adjusted *p*-values <0.01) between the subclasses where the majority were encountered in chromosome 6 (28%) and chromosome 11 (12%) (Table S5).

4 | DISCUSSION

Using clinical and DNA methylation array data from independent cohorts of adult-type *IDH*-mutant astrocytomas, we evaluate the prognostic relevance of DNA methylation-based subclassification in relation to the WHO 2021 CNS grading criteria and further examine potential prognostic markers (*CDK4*, *MYCN*, and *PDGFRA* amplifications) for *IDH*-mutant astrocytomas.

Our study demonstrates that the *IDH*-mutant astrocytoma methylation subclasses, obtained from the MNP classifier [13], can be used to refine the WHO 2021 CNS criteria for grading and prognostication of these tumors. The "lower grade" and "high grade" *IDH*-mutant astrocytoma methylation subclasses showed significant differences in survival outcomes and provided comparable risk stratification as the WHO 2021 CNS grading criteria. Cases where WHO 2021 CNS grade and the methylation subclass were in concordance (i.e., grade 2–3 tumors subclassified as "lower grade" or grade 4 tumors subclassified as "high grade") showed clear differences in clinical course, whereas the discordant cases (i.e., grade 3 tumors subclassified as "high grade" and grade 4 tumors subclassified as "lower grade") behave rather as an intermediate-risk group. This indicates that methylation-based subclassification can provide clinically important information for selected cases of *IDH*-mutant astrocytoma

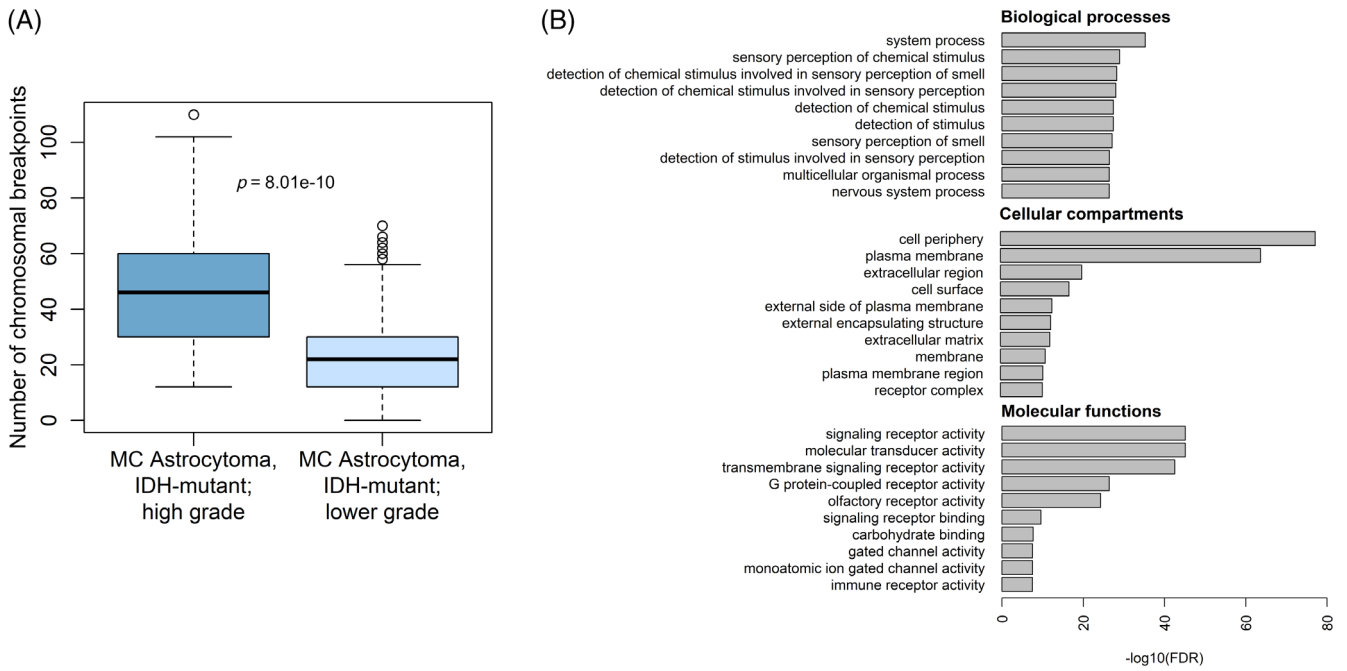

FIGURE 5 Chromosomal aberration and differential methylation analysis between the "high grade" and "lower grade" *IDH*-mutant astrocytoma methylation subclasses. (A) The "high grade" methylation subclass showed significantly higher numbers of chromosomal aberrations compared to the "lower grade" methylation subclass. (B) The 10 most significantly enriched pathways based on Gene Set Enrichment Analysis of differentially methylated positions ($\beta \geq 0.20$). Significance: $p < 0.01$. FDR, false discovery rate; MC, methylation class.

and can hence be used to refine WHO 2021 CNS grading criteria. Identification of these WHO 2021 CNS grade 3 tumors that are at "high risk" could be useful in the therapeutic management of these patients.

The "high grade" methylation subclass harbored increased levels of chromosomal breakpoints and worst clinical outcome compared with the "lower grade" subclass in *IDH* mutant astrocytomas. Elevated CNA levels in *IDH*-mutant astrocytomas have been shown to correlate with aggressive clinical behavior [19, 26–28], potentially explaining the clinical course of patients with the "high grade" methylation subclass.

Tesileanu et al. previously reported prognostic differences in grade 3 astrocytomas stratified by methylation subclasses, using an older version of the classifier [6], where a negative prognostic effect on survival was indicated for the "high grade" patients who did not receive adjuvant temozolomide treatment [29]. Further investigations with larger cohorts will be necessary to determine the overall prognostic role of the *IDH*-mutant astrocytoma methylation subclasses in this patient group.

The prognostic significance of *CDK4*, *MYCN*, and *PDGFRA* amplifications for *IDH*-mutant astrocytomas has been described in the literature [19, 30]. In our cohort, only *CDK4* and *MYCN* amplifications were associated with poor outcome in *IDH*-mutant astrocytoma patients, confirming the relevance of these molecular markers. Yet, stratification of the tumors by methylation subclasses outperformed *CDK4* and *MYCN* amplifications in predicting survival of the patients.

In this study, genome-wide DNA methylation array data was applied for quantitative assessment of *CDKN2A/B* homozygous deletion. The analysis of *CDKN2A/B* status using CNA profiles from methylation arrays is not commonly used in clinical practice, nonetheless, the suitability of this approach for *CDKN2A/B* analysis has been investigated in the literature [19, 29, 31].

Following the highlighted importance of DNA methylation profiling in CNS tumor diagnostics in WHO 2021 criteria, the use of methylation profiling in clinical practice is likely to increase. Both *CDKN2A/B* copy number status and methylation subclasses can be obtained from DNA methylation array data, which enables the prognostic assessment of these markers in clinical diagnostics in a single assay, sparing precious material and reducing analysis time and costs. Methylation analysis could, therefore, be preferred in situations with limited access to material such as in small biopsies.

5 | CONCLUSION

In conclusion, DNA methylation-based subclassification of *IDH*-mutant astrocytomas refines the WHO 2021 CNS grading system for these tumors. Because DNA methylation-based classification is increasingly used in routine clinical diagnostics, awareness of the implications of discordant information from WHO 2021 CNS grading and methylation-based subclassification is of practical importance.

AUTHOR CONTRIBUTIONS

Teresia Kling, Sandra Ferreyra Vega, and Helena Carén designed the study and Helena Carén coordinated it. Anna Dénes and Anna Lipatnikova provided data and Asgeir Store Jakola provided data and clinical input to the study. Sandra Ferreyra Vega and Medha Suman performed experimental procedures except for the histopathological evaluations, which were performed by Thomas Olsson Bontell. Sandra Ferreyra Vega, Medha Suman, Stina Lagerström, and Helena Carén generated data. Teresia Kling performed the data analysis and prepared the figures and tables with assistance from Sandra Ferreyra Vega and Helena Carén. Sandra Ferreyra Vega drafted the manuscript with input from Teresia Kling, Helena Carén, and Asgeir Store Jakola. All authors read and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

We thank UCL Genomics for EPIC array processing. The study was supported by the Swedish Cancer Society, the Swedish state under the agreement between the Swedish government and the county councils—the ALF-agreement (ALFGBG-965622 and ALFGBG-965828) and the Swedish Research Council.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw Infinium MethylationEPIC array data of IDH-mutant astrocytomas from the local cohort is publicly available at GEO (GSE175877).

ETHICS STATEMENT

This study was approved by the regional ethics committee in the Västra Götaland region in Sweden (Dnr 604-12, Dnr 1067-16, and T688-18). The study was performed in accordance with the Declaration of Helsinki.

ORCID

Helena Carén https://orcid.org/0000-0002-8584-555X

REFERENCES

1. Weller M, van den Bent M, Preusser M, Le Rhun E, Tonn J, Minniti G, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat Rev Clin Oncol. 2021; 18(3):170–86.
2. WHO Classification of Tumours Editorial Board. World Health Organization Classification of Tumours of the central nervous system. 5th ed. Lyon: International Agency for Research on Cancer; 2021.
3. Brat DJ, Aldape K, Colman H, Figrarella-Branger D, Fuller GN, Giannini C, et al. cIMPACT-NOW update 5: recommended grading criteria and terminologies for IDH-mutant astrocytomas. Acta Neuropathol. 2020;139(3):603–8.
4. Baylin SB, Jones PA. Epigenetic determinants of cancer. Cold Spring Harb Perspect Biol. 2016;8(9):a019505.
5. Jones P, Issa J, Baylin S. Targeting the cancer epigenome for therapy. Nat Rev Genet. 2016;17(10):630–41.
6. Capper D, Jones D, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555(7697):469–74.
7. Ferreyra Vega S, Olsson Bontell T, Corell A, Smits A, Jakola A, Caren H. DNA methylation profiling for molecular classification of adult diffuse lower-grade gliomas. Clin Epigenetics. 2021; 13(1):102.
8. Jaunmuktane Z, Capper D, Jones DTW, Schrimpf D, Sill M, Dutt M, et al. Methylation array profiling of adult brain tumours: diagnostic outcomes in a large, single centre. Acta Neuropathol Commun. 2019;7(1):1–18.
9. Priesterbach-Ackley LP, Boldt HB, Petersen JK, Bervoets N, Scheie D, Ulhøi BP, et al. Brain tumour diagnostics using a DNA methylation-based classifier as a diagnostic support tool. Neuropathol Appl Neurobiol. 2020;46(5):478–92.
10. Capper D, Stichel D, Sahm F, Jones D, Schrimpf D, Sill M, et al. Practical implementation of DNA methylation and copy-number-based CNS tumor diagnostics: the Heidelberg experience. Acta Neuropathol. 2018;136(2):181–210.
11. Pickles JC, Fairchild AR, Stone TJ, Brownlee L, Merve A, Yasin SA, et al. DNA methylation-based profiling for paediatric CNS tumour diagnosis and treatment: a population-based study. Lancet Child Adolesc Health. 2020;4(2):121–30.
12. Schepke E, Lofgren M, Pietsch T, Olsson Bontell T, Kling T, Wenger A, et al. DNA methylation profiling improves routine diagnosis of paediatric central nervous system tumours: a prospective population-based study. Neuropathol Appl Neurobiol. 2022; 48:e12838.
13. Molecular Neuropathology. Brain classifier 12.5 2022. Available from: https://www.molecularneuropathology.org/mnp
14. R CoreTeam. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2021. Available from: https://www.R-project.org/
15. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics. 2014;30(10):1363–9.
16. Tian Y, Morris TJ, Webster AP, Yang Z, Beck S, Feber A, et al. ChAMP: updated methylation analysis pipeline for Illumina BeadChips. Bioinformatics. 2017;33(24):3982–4.
17. Maksimovic J, Oshlack A, Phipson B. Gene set enrichment analysis for genome-wide DNA methylation data. Genome Biol. 2021; 22(1):173.
18. Hovestadt V, Zapatka M. conumee: enhanced copy-number variation analysis using Illumina 450k methylation arrays. R package version 190. 2015;4.
19. Shirahata M, Ono T, Stichel D, Schrimpf D, Reuss DE, Sahm F, et al. Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. Acta Neuropathol. 2018;136(1):153–66.
20. The Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. 2015;372(26):2481–98.
21. Ceccarelli M, Barthel F, Malta T, Sabedot T, Salama S, Murray B, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell. 2016; 164(3):550–63.
22. Wang LB, Karpova A, Gritsenko MA, Kyle JE, Cao S, Li Y, et al. Clinical proteomic tumor analysis C. Proteogenomic and metabolomic characterization of human glioblastoma. Cancer Cell. 2021;39(4):509–528.e20.
23. De Meyer T, Bady P, Trooskens G, Kurscheid S, Bloch J, Kros JM, et al. Genome-wide DNA methylation detection by MethylCap-seq and Infinium HumanMethylation450 BeadChips: an independent large-scale comparison. Sci Rep. 2015;5: 15375.

24. Kassambara A, Kosinski M, Biecek P, Fabian S. survminer: drawing survival curves using 'ggplot2' 2021. Available from: https://cran.r-project.org/web/packages/survminer/index.html
25. Therneau T. A package for survival analysis in R. R package version 3.4-0 2022. Available from: https://CRAN.R-project.org/package=survival
26. Mirchia K, Sathe AA, Walker JM, Fudym Y, Galbraith K, Viapiano MS, et al. Total copy number variation as a prognostic factor in adult astrocytoma subtypes. Acta Neuropathol Commun. 2019;7(1):92.
27. Richardson TE, Sathe AA, Kanchwala M, Jia G, Habib AA, Xiao G, et al. Genetic and epigenetic features of rapidly progressing IDH-mutant astrocytomas. J Neuropathol Exp Neurol. 2018;77(7):542–8.
28. Aoki K, Nakamura H, Suzuki H, Matsuo K, Kataoka K, Shimamura T, et al. Prognostic relevance of genetic alterations in diffuse lower-grade gliomas. Neuro Oncol. 2018;20(1):66–77.
29. Tesileanu CMS, van den Bent MJ, Sanson M, Wick W, Brandes AA, Clement PM, et al. Prognostic significance of genome-wide DNA methylation profiles within the randomized, phase 3, EORTC CATNON trial on non-1p/19q deleted anaplastic glioma. Neuro Oncol. 2021;23(9):1547–59.
30. Lee K, Kim SI, Kim EE, Shim YM, Won JK, Park CK, et al. Genomic profiles of IDH-mutant gliomas: MYCN-amplified IDH-mutant astrocytoma had the worst prognosis. Sci Rep. 2023;13(1):6761.
31. Kocakavuk E, Johnson KC, Sabedot TS, Reinhardt HC, Noushmehr H, Verhaak RGW. Hemizygous CDKN2A deletion confers worse survival outcomes in IDHmut-noncodel gliomas. Neuro Oncol. 2023;25(9):1721–3.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kling T, Ferreyra Vega S, Suman M, Dénes A, Lipatnikova A, Lagerström S, et al. Refinement of prognostication for *IDH*-mutant astrocytomas using DNA methylation-based classification. Brain Pathology. 2024;34(5):e13233. https://doi.org/10.1111/bpa.13233