Infratentorial IDH-mutant astrocytoma is a distinct subtype

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Abstract
Diffuse IDH-mutant astrocytic tumors are rarely diagnosed in the cerebellum or brainstem. In this multi-institutional study, we characterized a series of primary infratentorial IDH-mutant astrocytic tumors with respect to clinical and molecular parameters. We report that about 80% of IDH mutations in these tumors are of non-IDH1-R132H variants which are rare in supratentorial astrocytomas. Most frequently, IDH1-R132C/G and IDH2-R172S/G mutations were present. Moreover, the frequencies of ATRX-loss and MGMT promoter methylation, which are typically associated with IDH mutations in supratentorial astrocytic tumors, were significantly lower in the infratentorial compartment. Gene panel sequencing revealed two samples with IDH1-R132C/H3F3A-K27M co-mutations. Genome-wide DNA methylation as well as chromosomal copy number profiling provided further evidence for a molecular distinctiveness of infratentorial IDH-mutant astrocytomas. Clinical outcome of patients with infratentorial IDH-mutant astrocytomas is significantly better than that of patients with diffuse midline gliomas, H3K27M-mutant (p < 0.005) and significantly worse than that of patients with supratentorial IDH-mutant astrocytomas (p = 0.028). The presented data highlight the very existence and distinctiveness of infratentorial IDH-mutant astrocytomas that have important implications for diagnostics and prognostication. They imply that molecular testing is critical for detection of these tumors, since many of these tumors cannot be identified by immunohistochemistry applied for the mutated IDH1-R132H protein or loss of ATRX.

Keywords Infratentorial diffuse astrocytomas · IDH · ATRX · DNA methylation · Subtype

Introduction
Mutations in isocitrate dehydrogenase 1 (IDH1) or isocitrate dehydrogenase 2 (IDH2) genes are frequent alterations in diffusely infiltrating gliomas [6, 15, 18, 37, 38]. The revised 4th edition of the 2016 WHO classification of brain tumors established the presence of these mutations as a defining criterion for two types of diffuse gliomas of different WHO grades, diffuse astrocytic and oligodendrogliomas [24]. The two types are separated based on the presence or absence of a combined complete deletion of chromosomal arms 1p and 19q as the defining alteration for oligodendrogliomas, IDH-mutant and 1p/19q codeleted. Diffuse astrocytic tumors, on the other hand, lack such a co-deletion and include diffuse astrocytoma, IDH-mutant (WHO grade II), anaplastic astrocytoma, IDH-mutant (WHO grade III) and glioblastoma, IDH-mutant (WHO grade IV). The spectrum of IDH mutations is strikingly uneven in diffuse gliomas: in a series of more than 1000 of such tumors including 747 IDH-mutated samples, the IDH1-R132H type was found in 89% of cases; while, IDH1-R132C, R132S, R132G, R132L and
IDH2-R172K, R172M, R172W mutations were observed in 11% of these tumors [15]. In another study of 330 diffuse gliomas, 84% of 170 IDH-mutated samples harbored the canonical IDH1-R132H mutation and 16% carried other variants [38]. Additional frequent molecular characteristics of IDH-mutated astrocytic tumors are mutations or deletions in TP53 and ATRX [10]. Loss of ATRX protein expression in tumor cell nuclei of IDH-mutated, non-1p/19q-codeleted gliomas has frequently been observed with reported rates of 87–97% of cases [23, 30]. Among the potential consequences of loss of ATRX function is enabling of cellular immortalization via the alternative lengthening of telomeres (ALT) mechanism [3]. ALT and loss of ATRX are mutually exclusive with promoter-TERT mutation in diffuse gliomas, which is prevalent in oligodendroglioma, IDH-mutant and 1p/19q codeleted as well as glioblastoma, IDH-wildtype [4, 16, 21].

WHO denotes that IDH-mutant astrocytic tumors may be localized in any region of the CNS but the most common locations are the cerebral hemispheres especially the frontal lobe [24]. After initially regarded as practically being absent in diffuse brainstem gliomas, IDH mutations were later reported at remarkably low rates in infratentorial diffuse astrocytic tumors. Still, not much data have been published especially concerning the molecular characteristics and biological behavior of these mutations in infratentorial diffuse astrocytomas. According to the results of studies, only 23 IDH mutations have generally been reported in infratentorial astrocytomas comprising 14 brainstem and 9 cerebellar tumors with 9 mutations of the IDH1-R132H type and considerably 14 of the rare IDH1 (and IDH2) variants [7, 14, 19, 20, 26, 27, 29, 31]. Using a DNA methylation-based approach, we recently identified 6 IDH-mutant tumors in a series of 86 cerebellar tumors diagnosed as glioblastomas, of which harbored a rare IDH1 mutation increasing the need for further analyses [29].

The aim of the present study was to characterize a significantly larger series of such IDH-mutated diffuse infratentorial astrocytomas with respect to molecular and clinical parameters.

**Materials and methods**

**Sample selection and histology**

Formalin-fixed, paraffin-embedded (FFPE) tumor samples from the last 15 years of patients with diffuse infratentorial astrocytomas were collected from the archives of Neuropathology departments of Hannover Medical School (MHH), Heidelberg University Hospital, Charité – Universitätsmedizin Berlin, Frankfurt, Freiburg, Gießen/Marburg, Klinikum Bamberg, University of Greifswald and Göttingen according to the following inclusion criteria: (i) diffusely infiltrating astrocytic differentiation and (ii) tumor arising in infratentorial sub-compartments including brainstem, cerebellum and spinal cord. All diagnoses were adjusted to the 2016 WHO classification of brain tumors with at least 2 mitoses for indication of a WHO grade III and the evidence of necroses and/or microvascular proliferations for the diagnosis of glioblastoma [24]. Tumors with primary or additional supratentorial lesions according to the local clinical records were excluded. Patients’ gender, age (defined as age at initial diagnosis) and follow-up data were obtained from the local clinical records. In case of uncertainty, patients or their family physicians were contacted in individual cases by the local departments. Tissue collection and processing as well as data collection complied with local ethics regulations and approval. Reference sets were partly obtained from previous analyses [22, 30, 35].

**Immunohistochemistry**

Immunohistochemistry (IHC) was conducted for the detection of IDH1-R132H mutant protein and ATRX nuclear expression as previously described [30]. In brief, 4-μm-thick FFPE tissue sections were mounted on StarFrost Advanced Adhesive slides (Engelbrecht, Kassel, Germany) followed by drying at 80 °C for 15 min. IHC was performed on a BenchMark Ultra immunostainer (Ventana Medical Systems, Tucson, AZ, USA).

**DNA extraction, quantification and sequencing**

An automated Maxwell system (Promega, Fitchburg, Massachusetts, USA) was used for the extraction of DNA from FFPE tissue according to the manufacturer’s instructions. The Qubit dsDNA BR Assay kit was used for the determination of the DNA concentration (ThermoFisher Scientific, Carlsbad, California, USA). Target gene mutation hotspots for IDH1 and IDH2 were then amplified through PCR and analyzed by Sanger sequencing as previously described [15]. H3F3A and HIST1H3B analyses were performed by PCR and pyrosequencing as described elsewhere [17].

**DNA methylation profiling and chromosomal copy number profiling**

DNA methylation profiling was performed using the Illumina Infinium HumanMethylation450 or the Illumina Infinium HumanMethylation EPIC Kits as previously described and in accordance with the manufacturer’s instructions [11]. The DNA methylation array data were processed with the R/Bioconductor package minfi (version 1.20). The t-SNE plot was computed via the R package Rtsne using the 25,000 most variable CpG sites according to standard deviation
3000 iterations and a perplexity value of 5. For unsupervised hierarchical clustering analysis, we selected the 30,000 most variably methylated CpG sites across the dataset according to median absolute deviation. Pairwise similarity of samples was calculated using Euclidean distance. Clusters were then linked according to the Ward’s linkage method. Copy number variations (CNV) were calculated from the IDAT files using the R/Bioconductor package conumee including an additional baseline correction: (https://bioconductor.org/packages/release/bioc/html/conumee.html), https://github.com/dstichel/conumee). The CNV load was computed as previously described [29].

Promoter methylation status of MGMT was calculated according to the methylation data as described by Bady et al. [5]. Modifications include calculation of an individual confidence interval for MGMT promoter methylation for each probe. Samples in which the confidence interval included the cutoff value (0.358) were defined as not determinable.

Panel sequencing

Panel sequencing and data processing were conducted as previously described [33]. In brief, after shearing of DNA on a M220 Focused-ultrasonicator (Covaris, Woburn, Massachusetts, USA) and determination of DNA integrity and fragment size by the Bioanalyzer 2100 (Agilent, Santa Clara, California, USA), sequencing was performed on a NextSeq 500 instrument (Illumina, Carlsbad, USA). The data were automatically annotated using ANNOVAR software [36].

Statistical analyses

Overall survival (OS) was defined as the time between first surgery and death or the last follow-up. Patients who were still alive at last contact were censored. The survival curves were calculated using Kaplan–Meier method with 95% confidence intervals (95% CI). Log-rank test was applied to analyze differences of OS with a \( p \)-value \( \leq 0.05 \) (two sided) considered as statistically significant. For correlation analysis of clinicopathological features with molecular markers, we applied Pearson’s chi-square \( \left( \chi^2 \right) \) and Fisher’s exact test. Differences in CNV load between groups were tested for significance with unpaired \( t \)-tests. A \( p \)-value \( \leq 0.05 \) was considered as significant. The analyses were performed using the softwares IBM SPSS Statistics 25 (Armonk, NY) and SigmaPlot 14 (Systat Software, San Jose, CA).

Results

We identified a series of 43 infratentorial IDH-mutant astrocytomas from 42 patients entirely composed of tumors with unequivocal infratentorial localization including 12 WHO grade II, 18 WHO grade III and 12 WHO grade IV tumors (Fig. 1a). Recurrent tumors were included if the primary lesions were also localized in infratentorial regions. Tumors with primary or additional supratentorial lesions according to the local clinical records were excluded. None of the tumors included were associated with a known tumor syndrome. To compare the data of our study, we also compiled a cohort of 50 IDH-mutant astrocytomas with supratentorial hemispheric localization. Data regarding gender, age, histological grade, IDH-mutation type, ATRX IHC status, MGMT promoter status and methylation-based classification were collected.

Non-IDH-R132H mutations and male gender predominate in infratentorial astrocytomas

Twenty-six percent (26%) of tumors were only localized in the cerebellum, 53% were only in the brainstem and 21% were present in both brainstem and cerebellum (Fig. 1b). None of the tumors were localized in the spinal cord. Median age was 37 years, similar to that of supratentorial IDH-mutant astrocytomas (38), with a range of 1–68 years. There were almost twice as many male as female patients (1.8:1) (Table 1). Although no significant difference was achieved for the male–female ratio (M:F), there was a more pronounced male predominance in infratentorial tumors than in the supratentorial reference cohort, in which this ratio was 1.38:1 similar to tumor registry data [25]. The distribution of different IDH mutations in infratentorial tumors was strikingly different from their supratentorial counterparts. The typical IDH1-R132H mutation was present in only 24% of these tumors but in 82% of our supratentorial cohort and this difference was significant \( (p < 0.005) \) (Fig. 1c–e; Table 1 and supplementary Table 1). Accordingly, IDH mutation variants rarely present in supratentorial astrocytomas were frequently detected in infratentorial tumors, with IDH1-R132C (33%) and IDH1-R132G (28%) being the most frequent subtypes (Fig. 1c–d and Table 1). IDH2-R172S and IDH2-R172G mutations, which are extremely rare in supratentorial astrocytomas, were detected in 5% of our infratentorial tumors. Interestingly in a reference cohort of 42 samples entirely composed of supratentorial astrocytomas with non-R132H mutations, the M:F ratio was 2:1, almost similar to that of our infratentorial cases (1.8:1); while, this ratio was 1.28:1 among the IDH1-R132H mutant tumors from our supratentorial reference cohort confirming a high frequency of male patients in tumors with non-R132H mutations.

Reduced rates of ATRX-loss and MGMT promoter methylation in infratentorial astrocytomas

Loss of ATRX expression in tumor cell nuclei is a hallmark of IDH-mutant astrocytomas and was present in 94%
of cases in our supratentorial reference cohort. However, ATRX loss was significantly less frequent in infratentorial astrocytomas and was detectable in less than half of the assessable cases ($p < 0.005$) (Fig. 1c, d; Table 1). Interestingly, the M:F ratio was different in tumors with and without ATRX loss. Whereas this ratio was 0.8:1 in cases with retained ATRX expression, it increased to 2.7:1 in those with loss of ATRX and further increased to 3.3:1 in those with non-R132H mutation and loss of ATRX (supplementary Table 1). In $\chi^2$ and Fisher’s exact test, this effect was, however, not significant.

$MGMT$ promoter methylation is another molecular characteristic found in almost all IDH-mutant gliomas: All oligodendrogliomas IDH-mutant and 1p/19q codeleted [30] and about 96% of supratentorial IDH-mutant astrocytomas displayed $MGMT$ promoter methylation (Fig. 1e). In infratentorial IDH-mutant astrocytomas, this rate dropped to 56% and this decrease was significant ($p = 0.025$) (Fig. 1d). Importantly, in the reference cohort entirely composed of supratentorial tumors with rare IDH mutations, the frequencies of ATRX loss and $MGMT$ promoter methylation were 96% and 100%, respectively (Table 1), suggesting that the different frequencies of ATRX loss and $MGMT$ promoter methylation would associate with supra- or infratentorial localization but not with the type of IDH mutation. In line with this observation, no association was found between these two molecular features and IDH
mutation variant either among infratentorial tumors only or considering all tumors included in our study.

**H3F3A-K27M and IDH mutations may co-occur in infratentorial astrocytomas**

To get a deeper insight into the molecular profile of infratentorial IDH-mutant astrocytomas, we analyzed gene panel-sequencing data covering 130 brain tumor relevant genes for 10 infratentorial tumors. Two samples for which panel sequencing had been performed during routine diagnostic procedures harbored H3F3A-K27M mutations in addition to IDH1-R132C mutations and were diagnosed as diffuse midline gliomas, H3K27M-mutant WHO grade IV (DMG-K27M) accordingly. The mutant allele frequencies for IDH1-R132C and H3F3A-K27M were 21% and 27%, respectively, in one case and 37% and 21% in the other case suggesting that the majority of tumor cells carried both mutations. In DNA methylation profiling, however, both were classified into the methylation class “IDH glioma, subclass astrocytoma”. One patient was a 1-year-old male with a pontine tumor without MGMT promoter methylation who died after 14 months, probably the youngest patient with an IDH-mutant astrocytoma so far according to the literature. The other patient was a 16-year-old male with a brainstem tumor harboring an MGMT promoter methylation who died after 22 months. Interestingly, this tumor had a histologically benign appearance with pilocytic features and a very low mitotic index yet a diffusely infiltrating growth pattern (Supplementary Fig. 1). Of note, panel sequencing of blood-derived DNA of these two pediatric patients did not reveal a germline mutation.

As a consequence, we analyzed 17 additional infratentorial astrocytomas for H3F3A and HIST1H3B mutations through pyrosequencing but found no further mutations. All but one tumor analyzed using panel sequencing (9/10) harbored pathogenic mutations in TP53 and only 3 tumors showed truncating mutations in ATRX well associated with the ATRX expression status determined by IHC. No further recurrent mutations were found in the panel-sequencing analyses. Interestingly, no sample showed TERT promoter mutation including those with retained ATRX expression.

**DNA methylation profiling differentiates infratentorial from supratentorial tumors**

Global DNA methylation profiling proved to be a valuable tool for delineating biologically different tumor types and subtypes [11]. In a t-distributed stochastic neighbor embedding (t-SNE) analysis of 37 samples of the infratentorial cohort together with an extensive set of > 70,000 DNA methylation profiles including more than 80 molecularly defined classes of CNS tumors, non-CNS tumors and experimental data (i.e., cell lines, xenografts), infratentorial IDH-mutant astrocytomas cluster together with supratentorial IDH-mutant astrocytomas without clear separation (data not shown). Correspondingly, results for infratentorial IDH-mutant astrocytomas from the brain tumor classifier showed matching calibrated scores for the methylation classes “IDH glioma, subclass astrocytoma” and “IDH

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**Table 1 Clinico-pathological variables in supra- and infratentorial astrocytomas**

| Cohort                | Infratentorial | Reference-Supratentorial | Reference-Supratentorial IDH1-R132H | Reference-Supratentorial; non IDH1-R132H |
|-----------------------|----------------|--------------------------|-------------------------------------|------------------------------------------|
| n=                    | 42             | 50                       | 41                                  | 42                                       |
| Median age            | 37             | 38                       | 38                                  | 36                                       |
| Gender ratio          | 1.8:1          | 1.38:1                   | 1.28:1                              | 2:1                                      |
| WHO II                | 12 (29%)       | 18 (36%)                 | 18                                  | 14 (33%)                                 |
| WHO III               | 18 (42%)       | 19 (38%)                 | 10                                  | 20 (48%)                                 |
| WHO IV                | 12 (29%)       | 13 (26%)                 | 13                                  | 8 (19%)                                  |
| IDH1-R132H            | 24%            | 82%                      | 100%                                | –                                        |
| IDH1-R132C            | 33%            | 10%                      | –                                   | 45%                                      |
| IDH1-R132G            | 28%            | 4%                       | –                                   | 24%                                      |
| IDH1-R132S            | 5%             | 2%                       | –                                   | 14%                                      |
| IDH1-R132L            | 0%             | 0%                       | –                                   | 12%                                      |
| IDH2                  | 10%            | 2%                       | –                                   | 5%                                       |
| IDH2-R172S            | 5%             | 0%                       | –                                   | 0%                                       |
| IDH2-R172G            | 5%             | 0%                       | –                                   | 0%                                       |
| ATRX loss             | 47%            | 94%                      | 93%                                | 96%                                      |
| MGMT promoter methylation | 56% | 96%                       | 95%                                | 100%                                     |
glioma, subclass high grade astrocytoma”. Interestingly, however, in restricted sample sets, unsupervised t-SNE as well as unsupervised hierarchical clustering analyses almost perfectly separated infratentorial IDH-mutant astrocytomas not only from oligodendrogliomas, IDH-mutant and 1p/19q-codeleted, DMG-K27M and infratentorial pilocytic astrocytomas but also from supratentorial IDH-mutant astrocytomas (Fig. 2a–b). Further analyses restricted to supra- and infratentorial IDH-mutant astrocytomas samples from both localizations showed a tendency to split in two subgroups each (Fig. 2c). This pattern was consistently present using different computing parameters for analyses. Of note, neither supratentorial tumors localized in different lobes nor infratentorial tumors from different compartments revealed a tendency to form subgroups in t-SNE analyses (Supplementary Fig. 2c). Concerning histological WHO grade, tumors were distributed unevenly across the methylation subgroups. Tumors of WHO grade II and those of WHO grade IV accumulated in different subgroups of both supratentorial tumors and infratentorial tumors. This suggests that both supratentorial and infratentorial IDH-mutant astrocytomas form grade-related methylation subgroups. Interestingly, the two “low-grade” subgroups were more strongly separated from each other than the two “high-grade” subgroups possibly indicating that epigenetic alterations in the course of malignant progression influence and converge the epigenetic profiles in both supratentorial and infratentorial IDH-mutant astrocytomas. The positioning in the t-SNE map largely recapitulated the two methylation classes “IDH glioma, subclass astrocytoma” and “IDH glioma, subclass high-grade astrocytoma” from the brain tumor classifier except for 3/37 infratentorial astrocytomas including one WHO grade II tumor and the two cases with concurrent IDH1/H3K27M mutations and 5/50 supratentorial astrocytomas (Supplementary Table 1). Interestingly, the two samples with IDH1/H3K27M co-mutations clustered closely together and somewhat apart from the other
infratentorial IDH-mutant samples (Fig. 2c). Given the high frequency of non-IDH1-R132H mutations in infratentorial astrocytomas, we repeated the analyses with the reference cohort of supratentorial astrocytomas entirely composed of tumors with non-IDH1-R132H mutations. The result of t-SNE analyses showed that infratentorial tumors remain separated from their supratentorial counterparts with no tendency to form IDH-mutation-variant-specific subgroups (Fig. 2d and Supplementary Fig. 2b). Two grade-related subgroups were also observed for the supratentorial astrocytoma cohort with non-IDH1-R132H mutations which showed a perfect match with the results from the brain tumor classifier (Supplementary Table 1). Taken together, we conclude that DNA methylation profiles correlate with supra- and infratentorial localization and roughly with WHO grade but not with the type of IDH mutation.

**Chromosomal copy number profiles of infratentorial IDH-mutant astrocytomas reveal more frequent losses of 5p, 14q and 18q in low-grade tumors and less chromosomal alterations in high-grade tumors**

Next, we evaluated infratentorial IDH-mutant astrocytomas for chromosomal alterations using chromosomal copy number plots derived from the DNA methylation raw data. We generated summary copy number variation (CNV) plots according to the methylation subgroups identified including the supratentorial non-IDH1-R132H cohort (Infratentorial low grade, Infratentorial high grade, Supratentorial low grade, Supratentorial high grade; Supratentorial, non-IDH1-R132H, low grade; Supratentorial, non-IDH1-R132H, high grade). Importantly, none of the tumors showed a 1p/19q co-deletion.

The summarized CNV plots which show the frequency percentage of changes at a chromosomal location indicate that infratentorial IDH-mutant astrocytomas from the “low-grade” methylation subgroup exhibit more frequent losses of chromosomal arms 5p, 14q and 18q compared to tumors from the methylation subgroup “supratentorial low grade” (Fig. 3a–b). In comparison to astrocytomas from the subgroup “Supratentorial high grade”, those from the subgroup “Infratentorial high grade” showed a lower number of alterations but similarly demonstrated frequent loss of 9p including the CDKN2A/B locus (Fig. 3a–b). Curiously, chromosomal losses affecting at least 20% of the 18q arm were present in 35% of “Infratentorial low-grade” tumors but only in 12% of “Infratentorial high-grade” tumors (Supplementary Table 2). Of note, both chromosomal losses and gains of 5p and 18q were detectable in tumors of the “Supratentorial high-grade” and “Supratentorial, non-IDH1-R132H, high-grade” subgroups. Oncogene amplifications occurring in the “Infratentorial high-grade” subgroup only included MET (4/17) and EGFR (1/17) (Supplementary Table 1, Supplementary Fig. 3).

**Outcome of infratentorial IDH-mutant astrocytomas is intermediate between DMG H3K27M and supratentorial IDH-mutant astrocytomas**

IDH-mutant diffuse astrocytomas with infratentorial localization may show a similar radiological and histological appearance to DMG. DMG are often localized in infratentorial regions and are associated with a very poor clinical outcome; whereas supratentorial IDH-mutant astrocytomas have a significantly better outcome raising the question whether overall survival (OS) of patients with infratentorial IDH-mutant astrocytomas is significantly different. OS data were available for 22 patients with a median of 43.5 months. Fourteen patients had to be censored for the analysis. Kaplan–Meier analyses demonstrated that despite similar localization infratentorial IDH-mutant tumors were clearly associated with a longer OS compared to DMG (median 43.5 vs. 13 months, $p < 0.001$). In comparison to patients with supratentorial IDH-mutant astrocytomas, the OS of those with infratentorial IDH-mutant astrocytomas was shorter (median 43.5 vs. 74.5 months, $p = 0.007$) when the two cases with IDH1/H3K27M co-mutation were included (Fig. 4a). In case of exclusion of these two patients, there remained a trend toward worse outcome for infratentorial tumors but the difference to that of supratentorial IDH-mutant astrocytomas did not reach significance (Fig. 4b). Since most of the available survival data for infratentorial IDH-mutant astrocytomas were from tumors of the “low-grade” methylation subcluster, we performed an OS comparison of infratentorial “low-grade” tumors with two additionally compiled supratentorial IDH-mutant astrocytoma cohorts, representing the two grade-related methylation classes of supratentorial tumors. Patients with infratentorial “low-grade” tumors had significantly worse outcome than those with “Supratentorial, low grade” ($p = 0.013$). The outcome was, however, not different from “Supratentorial, high grade” tumors (Fig. 4c).

Considering all infra- and supratentorial IDH-mutant astrocytomas included in our study, no correlation was found between mutation subtype (R132H vs. non-R132H) and OS (Supplementary Fig. 4a). Furthermore, we evaluated patients’ outcome with infratentorial IDH-mutant astrocytomas regarding MGMT promoter methylation—a molecular marker considered to be associated with a more favorable prognosis in patients with supratentorial IDH-mutant astrocytomas. Such an association was, however, not found in our infratentorial IDH-mutant astrocytoma cohort, with the caveat, however, that the analysis could have been influenced by a rather low number of cases with available MGMT promoter status and OS data. Similarly, no correlation was observed between gender and patients’ outcome.
in infratentorial or supratentorial IDH-mutant astrocytomas (Supplementary Fig. 4b, c, d).

**Discussion**

Infratentorial IDH-mutant astrocytomas share with their supratentorial counterparts an astrocytic phenotype, a non-1p/19q-codeleted status and TP53 mutations. They also display a similar DNA methylation profile indicating a close relationship. The results of this study suggest, however, that primarily infratentorial IDH-mutant astrocytomas represent a distinct subtype of IDH-mutant astrocytoma.

Infratentorial astrocytomas show a predominance of non-canonical IDH-mutation variants that are rare in supratentorial tumors. This likely explains the paucity of infratentorial IDH-mutant tumors reported in the past since some studies used IDH1-R132H immunohistochemistry only. Even though all tumor-associated IDH1 and IDH2 mutations result in acquisition of a neo-enzymatic activity with the ability to convert α-ketoglutarate (α-KG) to the oncometabolite 2-hydroxylglutarate (2-HG), the spectrum of IDH mutation subtypes differs widely between different types of cancer [9]. In acute myeloid leukemia, the different IDH mutations are widely distributed [1]; while in all other types of cancers with IDH mutations, certain mutation variants occur much more often than others. For example, in angioimmunoblastic T-cell lymphoma exclusively IDH2-R172K and no IDH1 mutations at all have been reported [8]. Central chondrosarcoma and central and periosteal chondromas frequently carry IDH mutations but the IDH1-R132H variant was identified in only 17% of the mutated samples [2]. One hypothesis regarding these tumor-type specific differences relates to the fact that different IDH mutations are associated with significantly different levels of 2-HG and that high levels of intracellular 2-HG have been shown to
exhibit signs of toxicity, which is most likely cell-type and context dependent. The IDH1-R132H mutation results in lower concentrations of 2-HG compared to other mutational variants which has been suggested to be the reason of why this variant is predominantly selected in gliomas [28]. For infratentorial astrocytomas, this concept would suggest that

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**Fig. 4** Kaplan–Meier survival curves of patients with infratentorial IDH-mutant astrocytoma, diffuse midline glioma, H3 K27M-mutant (DMG-H3K27M) and supratentorial IDH-mutant astrocytoma. **a** The outcome of infratentorial IDH-mutant astrocytomas is significantly more favorable than DMG-H3K27M ($p < 0.005$) and poorer than supratentorial IDH-mutant astrocytomas ($p = 0.028$), if two cases with concurrent IDH and H3K27M mutations are considered. **b** After exclusion of these two patients, the difference of outcome between infratentorial IDH-mutant astrocytomas and DMG is still significant ($p < 0.005$), whereas there is a trend but no significance towards shortened overall survival in patients with infratentorial IDH-mutant astrocytomas compared to those of our supratentorial reference cohort ($p = 0.091$). **c** Comparison according to methylation subclusters shows poorer outcome of “infratentorial low-grade” tumors than “supratentorial low grade” ($p = 0.013$) but no difference between “infratentorial low grade” and “supratentorial high grade”
the cell of origin in infratentorial tumors is not susceptible to anti-proliferative effects of high 2-HG levels or that excessive 2-HG is more efficiently disposed in infratentorial structures.

The cell of origin is considered to have a major influence on the DNA methylation pattern of tumors [11, 32, 34]. Another important determinant may derive from mutations of an epigenetic regulator like IDH. However, different IDH-mutation variants do not associate with different DNA methylation profiles in supratentorial astrocytomas, suggesting that predominance of non-IDH1-R132H mutations in infratentorial astrocytomas does not explain the differences in DNA methylation, including the unusually high frequency of cases without MGMT-promoter methylation in infratentorial IDH-mutant astrocytoma.

Another unusual feature of infratentorial IDH-mutant astrocytomas is the distinctly low rate of ATRX loss compared to supratentorial IDH-mutant astrocytomas. Importantly, panel-sequencing results did not reveal TERT promoter mutations or mutations in DAXX in ATRX retaining IDH-mutant astrocytomas, which would be candidates for an alternative mechanism of immortalization. This may point to an unknown, ATRX-independent mechanism of immortalization in half of all IDH-mutant infratentorial astrocytomas.

Interestingly, the gender ratio in both supra- and infratentorial IDH mutant astrocytoma seemingly associates with the IDH-mutation variant because there are twice as many male as female patients among cases with non-IDH1-R132H mutations. In infratentorial astrocytomas, the ATRX status also interfered with the M:F ratio because more than three times more male than female patients are found in cases with loss of ATRX and a non-IDH1-R132H mutation but there is a balanced gender ratio in cases with retained expression of ATRX. ATRX belongs to tumor suppressor genes which may escape x-chromosomal inactivation in female brains [12]. Tumor formation in males may be facilitated by the need of inactivating only one copy of ATRX in contrast to the two potentially active copies of the gene in females. Interestingly, ATRX escape from inactivation was shown to occur heterogeneously across different brain regions [12]. Therefore, it is tempting to speculate that ATRX escape occurs more often or more efficiently in the cell of origin of infratentorial IDH-mutant astrocytomas compared to supratentorial tumors resulting in a more pronounced relative protection of females. However, additional studies will be necessary to clarify these phenomena.

Two infratentorial astrocytomas showed concurrent IDH1-R132C and H3F3A-K27M mutations representing a highly unusual finding considering the fact that these are defining mutations for different types of gliomas according to the WHO classification [24]. Awareness of this possible combination will predictably lead to the identification of more of such cases in the future. It will be interesting to analyze these tumors further with regard to the specific contribution of each mutation to tumor development. Even though more data from such rare tumors would be necessary for reliable conclusions, the short OS of the two cases in our series may suggest that the H3K27M mutation exhibits a dominant negative effect on the survival probability; whereas the global DNA methylation pattern only slightly differed from other IDH-mutant astrocytomas. This observation is of particular interest, since a methylation analysis alone would classify patients with such infratentorial astrocytomas with combined IDH and H3F3A mutations in the prognostically more favorable group of IDH mutant tumors, although the patients are more likely to have a worse prognosis, thus prompting the necessity to additionally analyze the H3K27M status of infratentorial IDH-mutant astrocytomas in the routine diagnostic workup.

The specific molecular features of infratentorial IDH-mutant astrocytomas have direct diagnostic implications, since testing for IDH-mutation using the IDH1-R132H-mutation specific antibody has a strongly reduced detection rate for these tumors. Also, the loss of ATRX expression in tumor cells which is a characteristic and diagnostically helpful feature of supratentorial IDH-mutant astrocytomas [13] is rather insensitive in infratentorial tumors. The immunohistochemical constellation of negativity for IDH1-R132H and retained expression of ATRX is very rare in supratentorial IDH-mutant astrocytomas (estimated < 1%) but frequently (36%) observed in infratentorial astrocytomas. Therefore, sequencing of IDH1/2 hotspot regions is mandatory for all infratentorial (diffusely infiltrating) astrocytic tumors. This may not be confined to tumors with a histological diffuse growth pattern, as at least one case of our series showed histological features of pilocytic astrocytoma. As discussed above also the presence of a H3K27M mutation does not necessarily exclude a concomitant IDH mutation.

In summary, infratentorial IDH-mutant astrocytomas should be recognized as distinct subtype and awareness of its existence and its specific characteristics have to be considered in diagnostic workflows.

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References

1. Abbas S, Lugthart S, Kavelaars FG, Schelen A, Koenders JE, Zeilemaker A, van Putten WJ, Rijneveld AW, Lowenberg B, Valk
PJ (2010) Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value. Blood 116:2122–2126. https://doi.org/10.1182/blood-2009-11-250878

2. Amary MF, Bacci C, Maggiani F, Damato S, Halai D, Berisha F, Pollock R, O’Donnell P, Grigoriadi A, Diss T et al (2011) IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. J Pathol 224:334–343. https://doi.org/10.1002/path.2913

3. Amorim JP, Santos G, Vinagre J, Soares P (2016) The role of ATRX in the alternative lengthening of telomeres (ALT) phenotype. Genes (Basel). https://doi.org/10.3390genes7080066

4. Arita H, Narita Y, Fukushima S, Tateishi K, Matsushita Y, Yoshida A, Miyakata Y, Ohno M, Collins VP, Kawahara N et al (2013) Upregulating mutations in the TERT promoter commonly occur in adult malignant gliomas and are strongly associated with total 1p19q loss. Acta Neuropathol 126:267–276. https://doi.org/10.1007/s00401-013-1141-6

5. Bady P, Scisciuro D, Diserens AC, Bloch J, van den Bent MJ, Marosi C, Dietrich PY, Weller M, Mariani L, Heppner FL et al (2012) MGMT methylation analysis of glioblastoma on the Infinium HumanMethylation27 BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. Acta Neuropathol 124:547–560. https://doi.org/10.1007/s00401-012-1016-2

6. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A (2008) Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 116:597–602. https://doi.org/10.1007/s00401-008-0455-2

7. Behling F, Steinhalber J, Tagatiba M, Bisdas S, Schittenhelm J (2015) IDH1 R132H mutation in a pilocytic astrocytoma: a case report. Int J Clin Exp Pathol 8:11809–11813

8. Cairns RA, Iqbal J, Lemonnier F, Kucuk C, de Leval L, Jais JP, Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A, Braun A, Krauss JK (2018) Cerebellar glioblastoma: a clinical series with contemporary molecular analysis. Acta Neuropathol (Wien) 160:2237–2248. https://doi.org/10.1007/s00701-018-3673-y

9. Ichimura K, Pearson DM, Kocialkowski S, Backlund LM, Chan R, Jones DT, Collins VP (2009) IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol 11:341–347. https://doi.org/10.1215/15228120-2009-0255

10. Ida CM, Lambert SR, Rodrigues FJ, Voss JS, Mc Cann BE, Seys AR, Halling KC, Collins VP, Giannini C (2012) BRAF alterations are frequent in cerebellar low-grade astrocytomas with diffuse growth pattern. J Neuropathol Exp Neurol 71:631–639. https://doi.org/10.1007/NEN.0b013e31825c448a

11. Javadi SA, Hartmann C, Walter GR, Braun R, Samii A (2018) IDH1 Mutation in brain stem glioma: case report and review of literature. Asian J Neurosurg 13:414–417. https://doi.org/10.4103/1793-5482.228540

12. Koelsche C, Sahm F, Capper D, Reuss D, Sturm D, Jones DT, Kool M, Northcott PA, Wiestler B, Bohmer K et al (2013) Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. Acta Neuropathol 126:907–915. https://doi.org/10.1007/s00401-013-1195-5

13. Korshunov A, Casalini B, Chavez L, Hielscher T, Sil M, Ryzhova M, Sharma T, Schrimpf D, Stichel D, Capper D et al (2019) Integrated molecular characterization of IDH-mutant glioblastomas. Neuropathol Appl Neurobiol 45:108–118. https://doi.org/10.1111/ana.12523

14. Leeper HE, Caron AA, Decker PA, Jenkins RB, Lachance DH, Giannini C (2015) IDH1 mutation, 1p19q codeletion and ATRX loss in WHO grade II gliomas. Oncotarget 6:30295–30305. https://doi.org/10.18632/oncotarget.4497

15. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Ellison DW, Noble M, Schold SC Jr, Miller AK, von Deimling A (2014) The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol Commun 2:78. https://doi.org/10.1186/s40478-014-0031-6

16. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Noble M, Schold SC Jr, Miller AK, von Deimling A (2014) The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol Commun 2:78. https://doi.org/10.1186/s40478-014-0031-6

17. Pusch S, Schweizer L, Beck AC, Lehmler JM, Weisert S, Balss J, Miller AK, von Deimling A (2014) D-2-Hydroxylutarate
producing neo-enzymatic activity inversely correlates with frequency of the type of isocitrate dehydrogenase 1 mutations found in glioma. Acta Neuropathol Commun 2:19. https://doi.org/10.1186/s40478-016-0327-7

29. Reinhardt A, Stichel D, Schrimpf D, Koelsche C, Wefers AK, Ebrahimia A, Sievers P, Huang K, Casalini MB, Fernandez-Klett F et al (2019) Tumors diagnosed as cerebellar glioblastoma comprise distinct molecular entities. Acta Neuropathol Commun 7:163. https://doi.org/10.1186/s40478-019-0801-8

30. Reuss DE, Sahm F, Schrimpf D, Wiestler B, Capper D, Koelsche C, Schweizer L, Korshunov A, Jones DT, Hovestadt V et al (2015) ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an “integrated” diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. Acta Neuropathol 129:133–146. https://doi.org/10.1007/s00401-014-1370-3

31. Reyes-Botero G, Giry M, Mokhtari K, Labussiere M, Idbaih A, Delattre JY, Laigle-Donadey F, Sanson M (2014) Molecular analysis of diffuse intrinsic brainstem gliomas in adults. J Neurooncol 116:405–411. https://doi.org/10.1007/s10937-013-1312-2

32. Rohrich M, Koelsche C, Schrimpf D, Capper D, Sahm F, Kratz A, Reuss J, Hovestadt V, Jones DT, Bewerunge-Hudler M et al (2016) Methylation-based classification of benign and malignant peripheral nerve sheath tumors. Acta Neuropathol 131:877–887. https://doi.org/10.1007/s00401-016-1540-6

33. Sahm F, Schrimpf D, Jones DT, Meyer J, Kratz A, Reuss D, Capper D, Koelsche C, Korshunov A, Wiestler B et al (2016) Next-generation sequencing in routine brain tumor diagnostics enables an integrated diagnosis and identifies actionable targets. Acta Neuropathol 131:903–910. https://doi.org/10.1007/s00401-015-1519-8

34. Sahm F, Schrimpf D, Stichel D, Jones DTW, Hielsercher T, Scheffzyk S, Okonechnikov K, Koelsche C, Reuss DE, Capper D et al (2017) DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis. Lancet Oncol 18:682–694. https://doi.org/10.1016/S1470-2045(17)30155-9

35. Shahraha M, Ono T, Stichel D, Schrimpf D, Reuss DE, Sahm F, Koelsche C, Wefers A, Reinhardt A, Huang K et al (2018) Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. Acta Neuropathol 136:153–166. https://doi.org/10.1007/s00401-018-1849-4

36. Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:e164. https://doi.org/10.1093/nar/gkq603

37. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol 174:1149–1153. https://doi.org/10.2353/ajpath.2009.080958

38. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ et al (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765–773. https://doi.org/10.1056/NEJMoa0808146

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