An Update on Pediatric Gliomas

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KEYWORDS

• Pediatric glioma • NTRK • EWSR1 • FGFR • HGNET-BCOR • BCOR-EP300

Key points

- Although the histology of pediatric gliomas can be indistinguishable from adult gliomas, their biology is distinct and it informs the diagnosis and prognosis.
- Gliomas with novel class-defining genetic events are emerging and can pose diagnostic challenges.
- Co-occurring genetic events in pediatric gliomas can be helpful in establishing a specific diagnosis and in guiding clinical management.

ABSTRACT

Pediatric gliomas are biologically distinct from adult gliomas. Although recent literature uncovered new genetic alterations, the prognostic implications of these discoveries are still unclear. This article provides an update on the histologic and molecular features with prognostic and/or therapeutic implications in pediatric gliomas.

OVERVIEW

Brain tumors are currently the leading cause of cancer-related death in pediatric patients.[1,2] Among them, low-grade gliomas are the most common (approximately 30% of all brain tumors) and are associated with significant morbidity, whereas high-grade gliomas are rare but incurable with currently available therapies.[3] Both low-grade and high-grade pediatric gliomas are clinically and biologically distinct from adult gliomas[4] and there is not enough published guidance regarding their histologic grading, specific classification, and outcomes.

This article covers the recent diagnostic, genetic, and prognostic discoveries in low-grade and high-grade pediatric gliomas. For practical purposes, most tumors that are classified by the World Health Organization (WHO) as grade I or II are discussed as low-grade gliomas, whereas tumors that are WHO histologic grade III or IV are generally discussed as high-grade gliomas. This article does not discuss the recent progress made in glioneuronal tumors, because that is the subject of a separate article in this issue.

PEDIATRIC LOW-GRADE GLIOMAS

Pediatric low-grade gliomas (PLGGs) are the most common brain tumors in children, representing 30% of all central nervous system (CNS) tumors. Despite their low mortality, they represent a significant challenge because of their high morbidity and multiple recurrences. PLGGs are clinically and biologically distinct from adult low-grade gliomas. The adult diffuse gliomas are characterized by *IDH* mutations, but these mutations are rare in children; almost all PLGGs are driven by genetic alterations that influence mitogen-activated protein kinase (MAPK) signaling pathway, and, among those, *BRAF* alterations are the most common.[5] This article reviews pilocytic astrocytoma, pilomyxoid astrocytoma, angiocentric glioma, pleomorphic xanthoastrocytoma, and emerging

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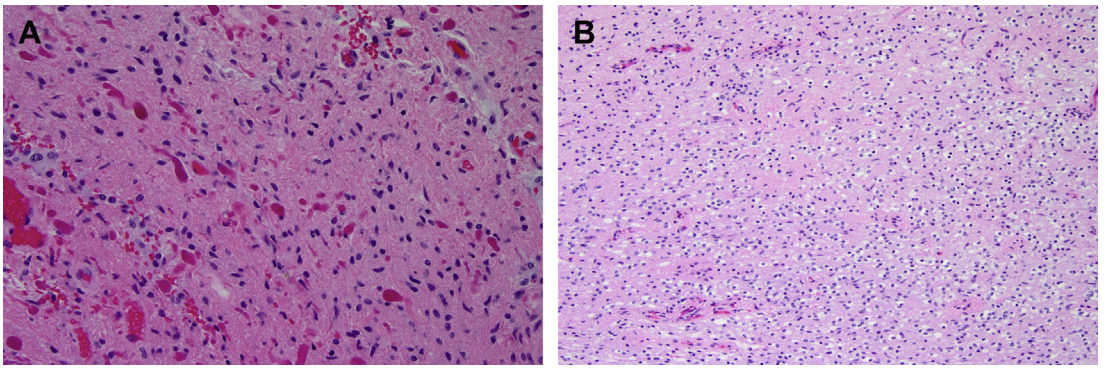

Fig. 1. PA. (*A*) PAs are composed of cells with round to oval mildly atypical nuclei and with piloid processes. Rosenthal fibers are frequent. (*B*) PAs sometimes show oligodendrogliomalike features.

entities with characteristic histology and molecular results.

PILOCYTIC ASTROCYTOMA

Among PLGGs, pilocytic astrocytoma (PA) is the most common and it represents 5.4% of all pediatric and adult gliomas.[2] It can occur anywhere along the neuraxis, but has a predilection for posterior fossa and, more specifically, cerebellum. Radiologically, PA appears as a well-demarcated solid and cystic tumor with contrast enhancement. On histology, PAs are composed of bipolar, mildly atypical cells with oval nuclei (**Fig. 1**A). They are usually biphasic tumors, with more cellular areas alternating with foci that are less cellular and have a myxoid background. Focal oligodendrocytelike morphology is common (**Fig. 1**B). Rosenthal fibers and eosinophilic granular bodies are usually present, and pseudocysts bordered by linear microvascular proliferation are common; in the setting of a PA, the microvascular proliferation should not prompt a concern for high-grade glioma. Occasionally, PAs have a mild or moderately high mitotic rate and proliferation index,[3] which is an accepted feature and not concerning for a higher-grade lesion.

Most PAs have activation of MAPK signaling through numerous alterations, of which the most common is a tandem duplication at 7q34 that results in *KIAA1549-BRAF* fusion, which is found in 98% of the posterior fossa cases.[6] The *BRAF* duplication can be observed through fluorescence in situ hybridization, array comparative genomic hybridization, or fusion assays. Although *BRAF* tandem duplication remains the most common event independent of location, more recent studies described numerous other genetic alterations that result in MAPK pathway activation: *BRAF V600E* mutation, *BRAF* intragenic deletions and insertions, *NTRK* fusions, and *FGFR1* and *KRAS* point mutations.[7–9]

PA is the CNS neoplasm most frequently associated with neurofibromatosis type 1 (NF1); in that setting, the most common location is the optic pathway.[10] Although NF1 is inherited in an autosomal-dominant pattern, approximately two-thirds of the cases of NF1 are caused by a sporadic mutation in the *NF1* gene encoding neurofibromin. Patients with NF1 have only 1 functional *NF1* gene. The second hit seen in PAs associated with NF1 syndrome occurs through point mutations or loss of heterozygosity.

Rarely, PA can be a symptom of Noonan syndrome, which is caused by germline mutations in MAPK pathway genes, the most common being *PTPN11*.[11]

The prognosis of PA is very good, with more than 95% of the patients being alive 5 to 10 years after surgical resection.[12] Histologic grade is usually maintained, and only rare cases develop anaplastic histology, usually after radiation or chemotherapy. Location and ability to resect the entire tumor are key factors in prognosis.[13]

PILOMYXOID ASTROCYTOMA

Pilomyxoid astrocytoma (PMA) affects mostly infants and young children, with a median age of 10 months. The most common location is the hypothalamus/optic pathway, although PMAs can be encountered anywhere in the neuraxis. The tumor was previously considered a distinct entity with a histologic WHO grade II, because of a possible less favorable prognosis than PA. Recent research showed that PMA and PA have similar biological backgrounds[14]; therefore, the 2016 WHO classification of the CNS tumors describes it as a histologic variant of PA and without a grade assigned. On histology, PMA has a pronounced myxoid background in which cells are arranged in a stellate, angiocentric fashion. Unlike PA, PMA lacks Rosenthal fibers and eosinophilic granular bodies. The literature hypothesizes that their more aggressive behavior is caused by the young age at presentation and tumor location, which often makes a complete resection impossible.

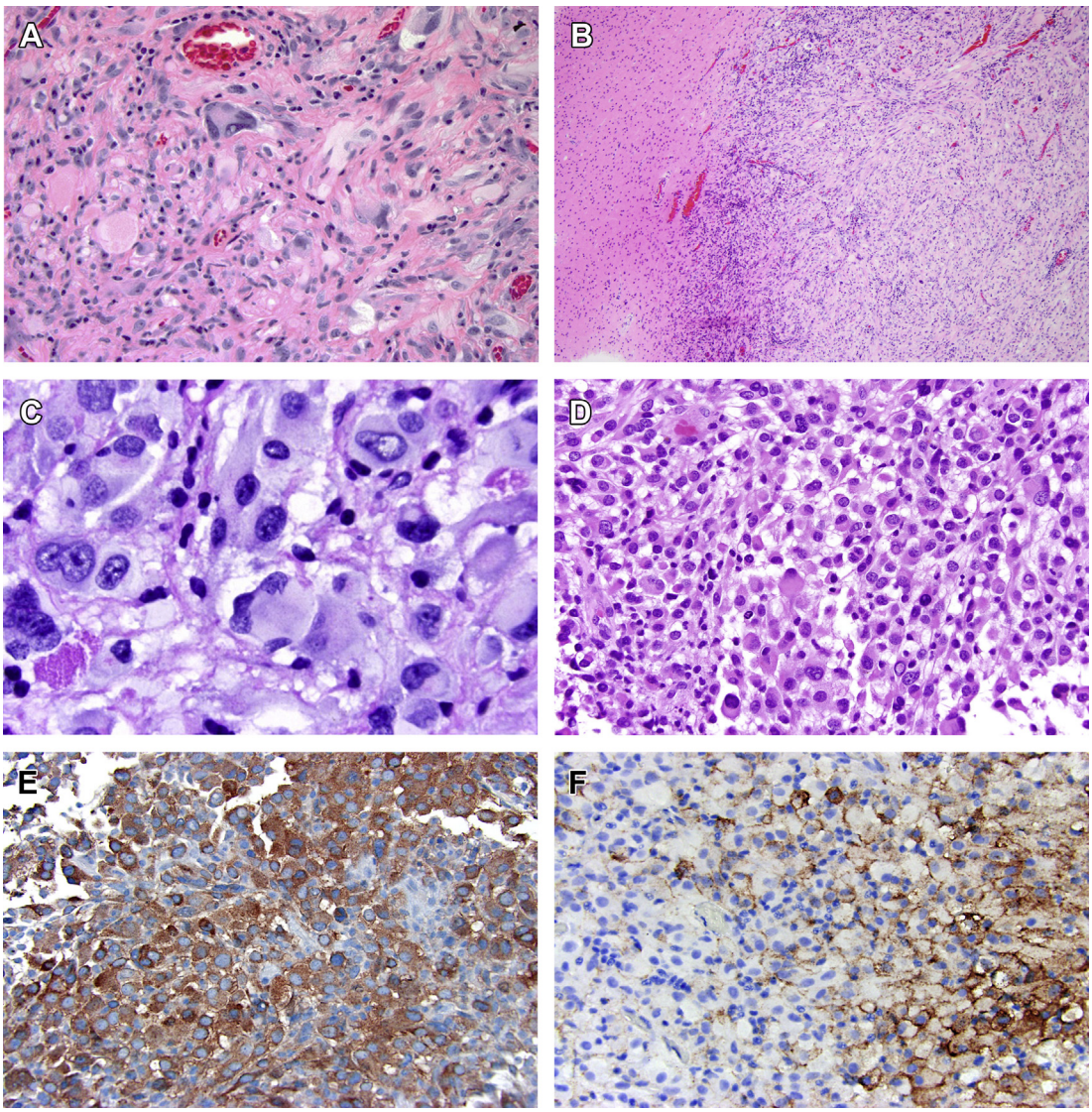

Fig. 2. PXA. (*A*) PXA is composed of spindle and bizarre cells, of which some have lipidized cytoplasm. Lymphocytes can be present around the vessels and admixed with the tumor (hematoxylin-eosin [H&E]). (*B*) Usually there is a sharp demarcation between PXA and surrounding brain (H&E). (*C*) Eosinophilic granular bodies are a common finding in PXA, and the periodic acid–Schiff (PAS) special stain can highlight them (PAS). (*D*) An example of epithelioid PXA composed of cells with abundant eosinophilic cytoplasm, eccentric nuclei, prominent nucleoli, and distinct cellular borders (H&E). (*E*) BRAF V600E immunostain is positive in most PXAs. (*F*) Epithelioid PXAs show patchy epithelial membrane antigen (EMA) immunoexpression.

PLEOMORPHIC XANTHOASTROCYTOMA

Pleomorphic xanthoastrocytoma (PXA) is a solid, noninfiltrative, epileptogenic tumor of childhood and young adulthood. It usually occurs in the superficial temporal lobe, involving the cortex, superficial white matter, and sometimes leptomeninges; however, it can occur anywhere in the CNS. On histology, PXA is composed of glial cells with spindled morphology admixed with bizarre, sometimes multinucleated, cells (**Fig. 2**A, B). Xanthomatous changes and scattered eosinophilic granular bodies are common (**Fig. 2**C), and the tumor creates a characteristic reticulin-rich network around individual cells (not shown). Although PXA is regarded as a WHO grade II tumor with good prognosis after complete surgical resection, occasional cases have increased mitotic rate (4 or more per 10 high-power fields) and necrosis, prompting a WHO grade III classification. Sometimes anaplastic PXAs can have epithelioid morphology, making it difficult to distinguish from epithelioid

glioblastomas (**Fig. 2**D). In such instances, focal features of classic PXA that include eosinophilic granular bodies indicate an anaplastic PXA.

Approximately 50% to 78% of PXAs have *BRAF* point mutations, the most common being *BRAF V600E* immunostain is used in clinical practice as a surrogate for the specific mutation (**Fig. 2**E).[15] Another common alteration in PXA is loss of chromosome 9, encountered in 50% of the cases.[16] Homozygous deletion of *CDKN2A/2B* is present in 60% of PXAs. A recent study by Phillips and colleagues[17] showed *TERT* alterations (hotspot mutations and amplifications) in 7 of 15 anaplastic PXAs included in the cohort.

The diagnosis of PXA can be challenging, especially when the histology is anaplastic or the tumor does not harbor a *BRAF V600E* mutation. In such situations, in order to distinguish these cases from other gliomas and particularly from glioblastoma, comprehensive molecular characterization is needed. Phillips and colleagues[18] described *ATG7-RAF1* and *NRF1-BRAF* fusions in 2 cases of anaplastic PXA without *BRAF V600E*.

One of the challenges in practice is differentiating an anaplastic epithelioid PXA from an epithelioid glioblastoma; the radiographic features, histology, and molecular background of these tumors are often indistinguishable. Both are solid tumors composed of large cells with abundant cytoplasm and nuclei with prominent nucleoli. Like PXA, epithelioid glioblastoma tends to have a superficial location and to involve leptomeninges.[19] They both have BRAF V600E mutations and, interestingly, focal EMA immunopositivity (**Fig. 2**F). A recent study by Korshunov and colleagues[20] showed that epithelioid glioblastomas stratify into established diagnostic categories on clustering methylome analysis, with a subset of pediatric glioblastomas clustering with PXAs and having a good prognosis.

ANGIOCENTRIC GLIOMA

Angiocentric glioma was first described in 2005 by Wang and colleagues[21] as an infiltrative low-grade epileptogenic cerebral glioma showing T2 hyperintensity on MRI. The tumors are composed of bipolar cells with elongated nuclei, which infiltrate the brain matter and show angiocentricity (**Fig. 3**). Glial immunomarkers are positive. Mitoses are difficult to find, and necrosis and microvascular proliferation are usually not encountered. In the study by Wang and colleagues,[21] all but 1 patient were free of disease after surgical resection, with a follow-up ranging from days to 62 months. Recently, a *MYB-QKI* gene fusion was identified as the defining genetic alteration in most angiocentric gliomas.[22] The 2016 WHO definition of angiocentric glioma is a grade I cerebral tumor; however, in 2017, 3 almost simultaneous articles described angiocentric gliomas arising in the brainstem, challenging the WHO definition.[23–25]

LOW-GRADE PEDIATRIC GLIOMAS WITH NEWLY DISCOVERED MOLECULAR ALTERATION

Diffuse low-grade gliomas in the pediatric population are difficult to classify in a specific category; they challenge the WHO grading system, which is primarily applicable to adult diffuse gliomas.[3] Pediatric diffuse low-grade gliomas are usually negative for *IDH*, *ATRX*, and *p53* mutations, and usually do not have 1p/19q whole arm codeletion. Application of comprehensive clinical molecular studies leads

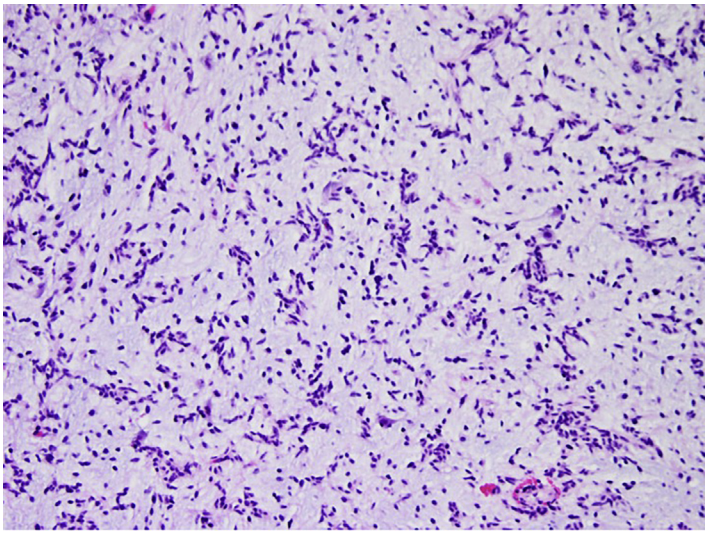

Fig. 3. Angiocentric glioma. Angiocentric glioma is an infiltrative low-grade epileptogenic neoplasm with elongated, mildly atypical cells that display angiocentric arrangement in a myxoid background stroma.

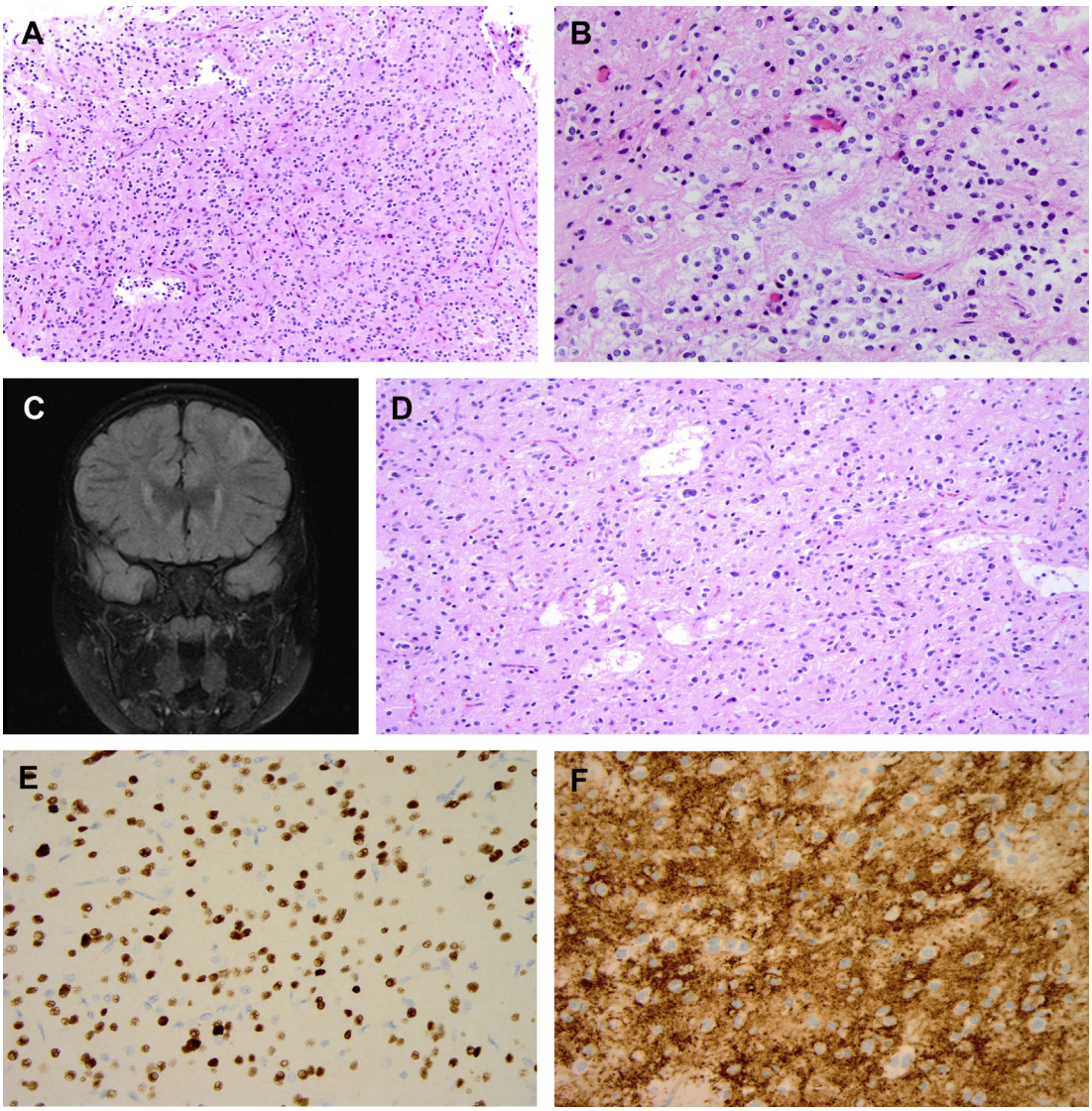

Fig. 4. (*A*, *B*) A diffuse pediatric glioma with *FGFR3-TACC3* rearrangement; it is composed of oligodendrocytelike cells with round and oval nuclei, perinuclear clearing, and occasional delicate vessels (H&E). (*C*–*F*) A polymorphous low-grade neuroepithelial tumor of youth with *FGFR2-CTNNB1* rearrangement in a 17-year-old patient. (*C*) T2 fluid-attenuated inversion recovery MRI showing an infiltrative superficial tumor in the left frontal lobe. (*D*) The glioma is composed of cells with mild-to-moderate nuclear atypia, occasional microcysts and delicate vascular channels (H&E). (*E*) OLIG2 immunostain is diffusely expressed and (*F*) the tumor cells are positive for EMA immunoexpression. Mitoses, microvascular proliferation, and necrosis are absent.

to the discovery of diffuse low-grade gliomas with *FGFR* point mutations and fusions,[26] *NTRK* fusions,[27] as well as more rare fusions involving unexpected genes, such as *EWSR1* and *BCOR*.[28,29]

Aside from PA with *FGFR* point mutations (mostly p.N546K and p.K656E), *FGFR1* point mutations and *FGFR* fusions can be present in a subset of pediatric diffuse gliomas in any location in the neuraxis. The most common fusions are *FGFR1-TACC1*, *FGFR2-CTTNA3*, and *FGFR3-TACC3* fusions. Another common *FGFR* alteration in pediatric gliomas is *FGFR1 TKD*. In one of the authors' experience (S.A.), *FGFR1-TACC1* and *FGFR3-TACC3* fusions can be seen in both low-grade and high-grade diffuse pediatric gliomas (**Fig. 4**A, B).

In the authors' experience, gliomas with *FGFR2-CTTNA3* fusions have common clinical, radiologic, histologic, and molecular features. Patients usually present with seizures and a hemispheric mass (**Fig. 4**C); microscopic examination shows an infiltrative glial neoplasm with cells with speckled chromatin and perinuclear clearing (oligolike features), a

rich network of delicate vascular channels, and scattered calcifications (Fig. 4D). The tumor cells are diffusely positive for glial markers (Fig. 4E) and sometimes they can show diffuse CD34 immunopositivity (Fig. 4F), similar to the tumors described by Huse and colleagues[30] as polymorphous low-grade neuroepithelial tumor of the young (PLNTY). In this study of 10 tumors diagnosed as PLNTY with a follow-up ranging from 12 to 89 months, a single patient had new seizures and progression 36 months after gross total resection; the rest of the patients were without evidence of disease after surgical management, resembling the clinical course of a WHO grade I glioma. The tumors that did not have *FGFR2-CTNNA3* fusion had *BRAF V600E* mutation, or *FGFR2-KIAA1549* fusions; they all had a similar behavior.

Alterations in tropomyosin-related kinase (TRK) genes are found mostly in adult and infantile glioblastomas.[31,32] The authors have encountered in practice pediatric diffuse gliomas with *NTRK* rearrangements of all grades; their histology seems to vary from case to case and there seems to be no correlation between histology and a particular *NTRK* gene or fusion partner, although specific studies on larger cohorts of *NTRK*-rearranged gliomas are needed for meaningful conclusions. In order to show the range of histologic features of *NTRK*-rearranged gliomas, 2 cases, 1 with low-grade and 1 with high-grade histology, are shown in Fig. 5. At present, there are no well-defined studies describing the outcome of patients with *NTRK*-fused gliomas. Although the use of pan-TRK antibody is becoming widespread in mesenchymal tumors, its use in neuropathology practice needs to take into consideration that normal brain expresses TRK proteins and positivity is expected in normal circumstances, hence the immunosurrogate is not an objective indicator of rearrangement in gliomas.

With the increased number of molecular modalities, fusions between *EWSR1* and novel partners have been described in soft tissue tumors. Alterations in *EWSR1* were not described in gliomas until 2019, when Siegfried and colleagues[28] reported a case of an intraventricular glioneuronal tumor with *EWSR1-PATZ1* fusion (exon 8 of *EWSR1* and exon 1 of *PATZ1*). The histology of the described tumor is low grade with areas of increased cellularity, pleomorphism, and a rich network of hyalinized vessels. The tumor is positive for OLIG2, and focally positive for glial fibrillary acidic protein (GFAP) and synaptophysin. Methylation analysis indicated that the tumor is distinct from all other well-described entities. In addition, 3 large-scale sequencing studies on pediatric and adult gliomas contain 3 gliomas with *EWSR1-PATZ1* fusion, of which 1 is described as high grade.[33–35] However, these studies do not comment on histology and outcomes and do not speculate on whether these tumors represent a new entity or not. The senior author of this article (S.A.) encountered a case of an intraventricular glioma with an *EWSR1-PATZ1* fusion (Fig. 6). The histology was similar to that described by Siegfried and colleagues[28] for case 1: the tumor had increased cellularity and pleomorphism and a rich vascular network, but it lacked microvascular proliferation, mitoses were rare, and there was no necrosis, prompting a diagnosis of low-grade glioma. Unlike Siegfried and colleagues'[28] case, this case did not have neuronal features.

GLIOMAS WITH ISOCITRATE DEHYDROGENASE MUTATIONS IN PEDIATRIC PATIENTS

The WHO groups diffuse infiltrating gliomas based on the presence or absence of isocitrate dehydrogenase (*IDH*) mutations, with *IDH*-mutated tumors in either diffuse astrocytoma (which have co-occurring *TP53* and *ATRX* mutations) or oligodendroglioma (defined by the presence of *IDH* mutation and 1p/19q whole arm codeletion) categories. In practice, the diagnostic work-up for diffusely infiltrating gliomas involves (1) immunohistochemistry for IDH1 R132H, p53, and ATRX; (2) analysis of 1p/19q status by fluorescent in situ hybridization, array comparative genomic hybridization, or polymerase chain reaction; and, if available, (3) targeted next-generation sequencing to identify molecular oncogenic driver mutations and fusions.[36]

Low-grade infiltrating gliomas in adults are nearly ubiquitously driven by *IDH1* or *IDH2* mutations,[3] whereas *IDH* mutations are exceedingly uncommon in low-grade gliomas of childhood. A landmark study profiling whole-genome sequencing of 151 low-grade gliomas from 149 patients found only a single *IDH*-mutated tumor in a 15-year-old patient.[37] Ferris and colleagues[38] describe a short series of 3 young patients (9, 9, and 7 years old) with diffuse astrocytomas with IDH mutations. This study shows the possible presence of IDH mutations in children younger than 10 years; interestingly, 2 of the patients in this series had intact ATRX expression and lacked *ATRX* and *TERT* promoter mutations, suggesting that some *IDH*-mutant gliomas in children might be distinct from their adult counterpart.

The youngest patient with an *IDH1*-mutant diffuse astrocytoma seen at our institution was a 6-year-old patient with a frontal lobe glioma; histologically, the tumor was indistinguishable from an adult-type diffuse glioma WHO grade II (Fig. 7) and targeted exome sequencing confirmed the *IDH1(R132H)* mutation, but did not reveal any *ATRX*, *TP53*, *TERT* mutations or 1p/19q whole

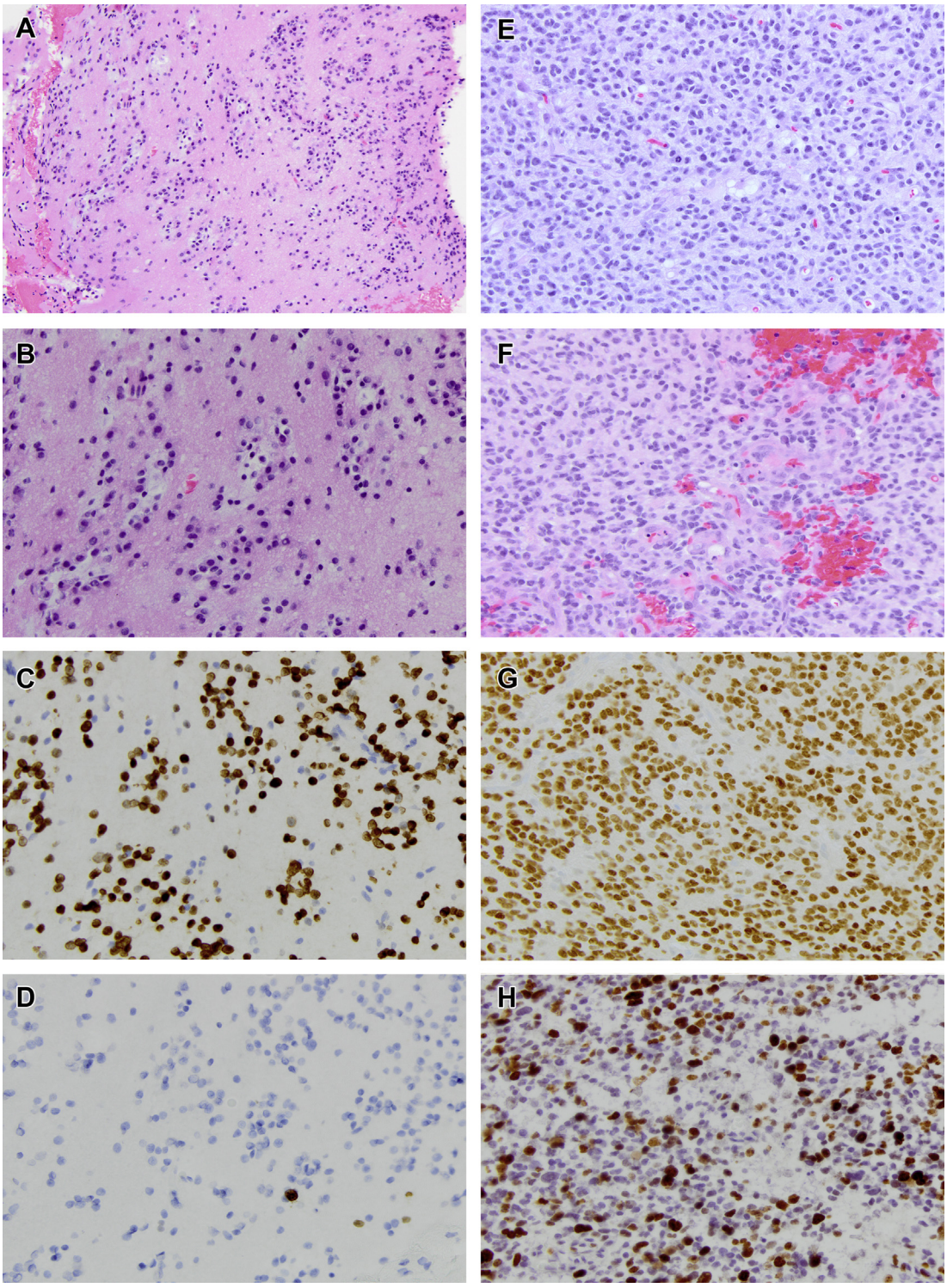

Fig. 5. *NTRK*-rearranged gliomas. (*A–D*) A low-grade NTRK-rearranged low-grade glioma in the insula of a 21-year-old patient. (*A*) The infiltrative glioma is arranged in hypercellular nests admixed with areas that are less cellular (H&E). (*B*) The cells have oligodendrocytelike features and mild nuclear atypia. No mitoses, necrosis, or microvascular proliferation were seen (H&E). (*C*) OLIG2 immunostain was diffusely positive and (*D*) the Ki67 proliferation-labeling index was 1% to 2%. (*E–H*) An NTRK-rearranged high-grade glioma in the parietal lobe of a 4-year-old patient. (*E, F*) show a densely cellular neoplasm composed of cells with oval, moderately atypical nuclei. Occasional mitoses are present, and foci containing microvascular proliferation were easily identified (H&E).

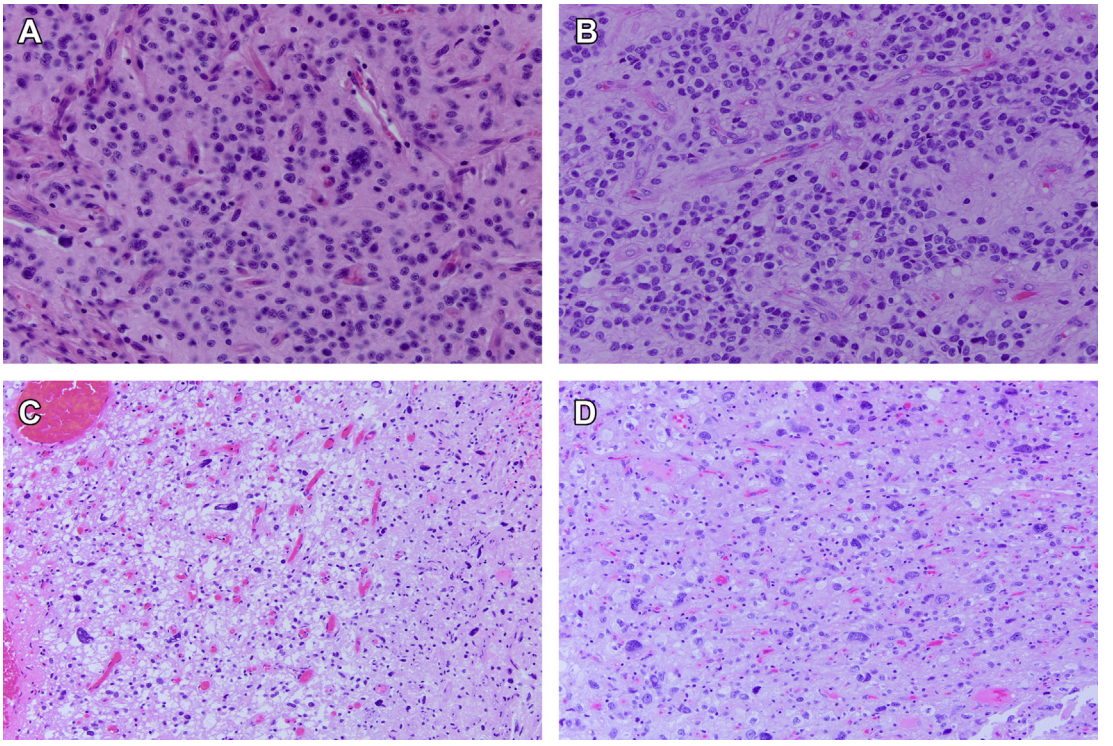

Fig. 6. Glioma with *EWSR1-PATZ1* rearrangement. The histology of this case was highly variable, with oligodendrocytelike areas and a rich network of delicate vessels (*A*) admixed with areas in which the cells were monotonous and had a vague pseudorosette pattern (*B*). Some foci resembled low-grade glioneuronal tumors with a myxoid background stroma, spindled-appearing cells, some with nuclear atypia (*C*) and scattered ganglionlike cells (*D*).

arm codeletion. This case supports the conclusion in Ferris and colleagues[38] study.

PEDIATRIC HIGH-GRADE GLIOMAS

Pediatric high-grade gliomas have similar histologic features to their adult counterparts, but they are biologically different. In recent years there have been major advances in genetic profiling of pediatric high-grade gliomas, which led to separation of some entities from histologically similar adult and pediatric counterparts. Most pediatric high-grade gliomas are primary, and transformation from a low-grade to a high-grade glioma is estimated at less than 10% in children.[38]

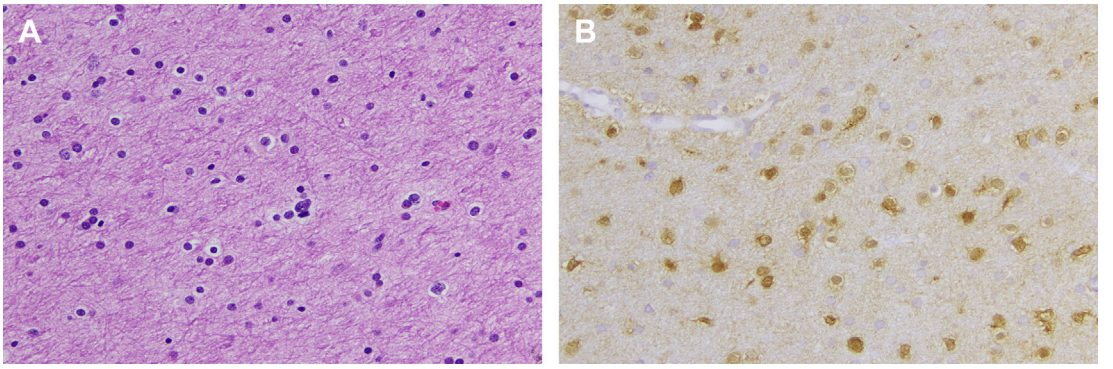

Fig. 7. Diffuse glioma, *IDH1 (R132H)* mutant. (*A*) A section of the white matter shows occasional minimally atypical nuclei; overall the histology is similar to that of a mildly reactive white matter. (*B*) An IDH1 immunostain highlights all the mutant cells.

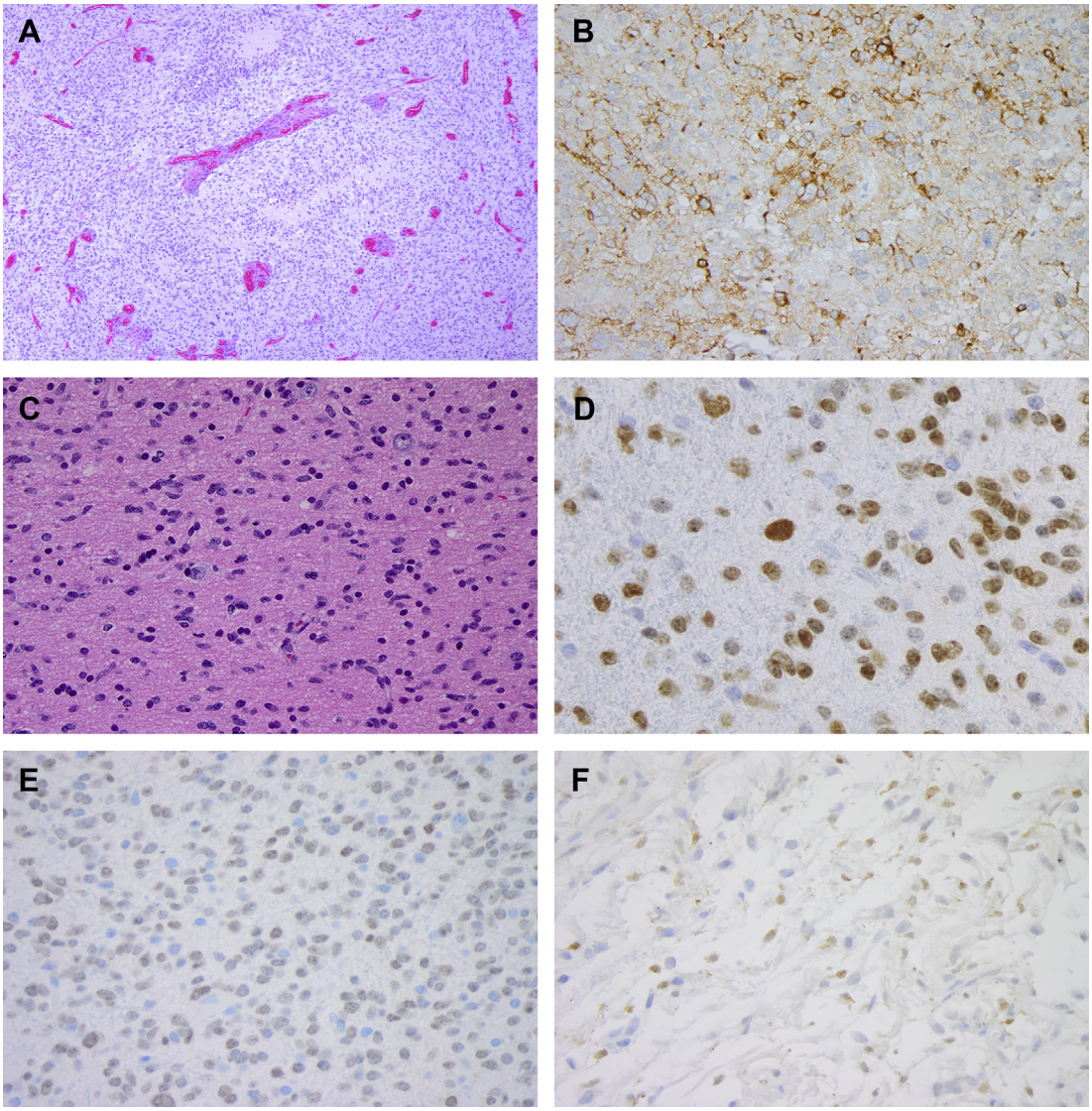

Fig. 8. High-grade glioma. (*A*) A congenital glioblastoma with *ALK1* rearrangement: increased cellularity, microvascular proliferation, and necrosis are present. (*B*) ALK1 immunostain shows significant positivity. (*C*) Representative diffuse midline glioma with occasional mitoses. (*D*) H3K27M immunostain shows crisp nuclear staining in most tumor cells, in keeping with *H3K27M* mutation; this patient was confirmed to have an *H3F3A* mutation. (*E*) Pale nuclear H3K27M immunostaining that was observed in a diffuse midline glioma with confirmed *HIST1H3B* mutation. (*F*) Granular cytoplasmic staining in some of the cells in a midline glioma confirmed negative for histone H3 mutations.

CONGENITAL AND INFANTILE HIGH-GRADE GLIOMAS

Among pediatric high-grade gliomas, congenital and infantile glioblastomas are sometimes biologically distinct from those occurring beyond this age. Congenital high-grade gliomas are often detected at prenatal ultrasonography as an echogenic, usually hemispheric large mass that produces midline shift and hydrocephalus. Genetically, these tumors are distinct from high-grade glioma in childhood and adulthood and they tend to have better outcomes; although they can harbor loss of *PTEN* and *TP53* mutations, *EGFR* amplification, *IDH*, and histone mutations have not been described.[39] Recent publications and clinical observations show enriched *MET*/*ALK*/*ROS1*/*NTRK* alterations in a subset of congenital and infantile high-grade gliomas.[31,34] This discovery is particularly important for management, because *ALK1* inhibitors are used in some adult malignancies and experimental studies on glioblastoma lines exist.[40] **Fig. 8**A, B shows an *ALK*-rearranged congenital glioblastoma.

Beyond infancy, alterations in histone H3 (most commonly *H3F3A*, *HIST1H3B*, and *G34V/R*), *BRAF*, *NTRK*, and *BCOR* are known oncogenic drivers in pediatric diffuse high-grade gliomas.

HISTONE H3-MUTATED GLIOMAS

Diffusely infiltrating tumors arising in midline structures often harbor *H3K27M* mutations, and this group of tumors is associated with aggressive biological activity and poor clinical outcome.[41,42] This group includes most diffuse intrinsic pontine gliomas (DIPGs), many midline glioblastomas, and a small proportion of histologically low-grade diffuse astrocytomas located in midline structures.[37,43] As a result, in 2016, these tumors became recognized as a distinct grade IV entity by current WHO guidelines (diffuse midline glioma, K3K27M mutant, WHO grade IV) regardless of their histologic appearance.[3] A classic case of a diffuse midline glioma with H3K27M mutation and the patterns of expression of the H3K27M antibody are shown in **Fig. 8**C–F.

Pediatric midline gliomas harbor mutually exclusive mutations in 1 of 2 genes: the histone H3.3 gene *H3F3A* or H3.1 *HIST1H3B*.[44] The *H3K27M* mutations are primarily found in midline gliomas arising in the brainstem, spinal cord, and thalamus, although Zhang and colleagues[37] observed 1 case of low-grade diffuse astrocytoma with *H3K27M* mutation located in the cerebral hemisphere. Distinct histologic features and differences in clinical outcome between brainstem tumors with *H3.3K27M* and *H3.1K27M* have been described, with worse prognosis in the *H3.3K27M* group.[45]

In contrast, G34V/R mutations of *H3F3A* occur almost exclusively in hemispheric pediatric high-grade gliomas, and patients experience better overall survival.[46] Again, there are notable differences in clinical and molecular characteristics between K27M and G34R/V mutation, suggesting that these tumors have unique oncogenic derivation and likely represent distinct diseases.[47]

H3K27M mutations are not specific to diffuse gliomas, and have been identified in posterior fossa ependymoma,[48,49] PA,[50,51] and gangliogliomas.[52] The authors have encountered 1 such case of a ganglioglioma with *BRAF V600E* and *H3K27M* mutation located in the cervical spine of a 7-year-old patient who presented with excoriation disorder and asymmetric forearm muscle bulk (**Fig. 9**). Pages and colleagues[52] reported 5

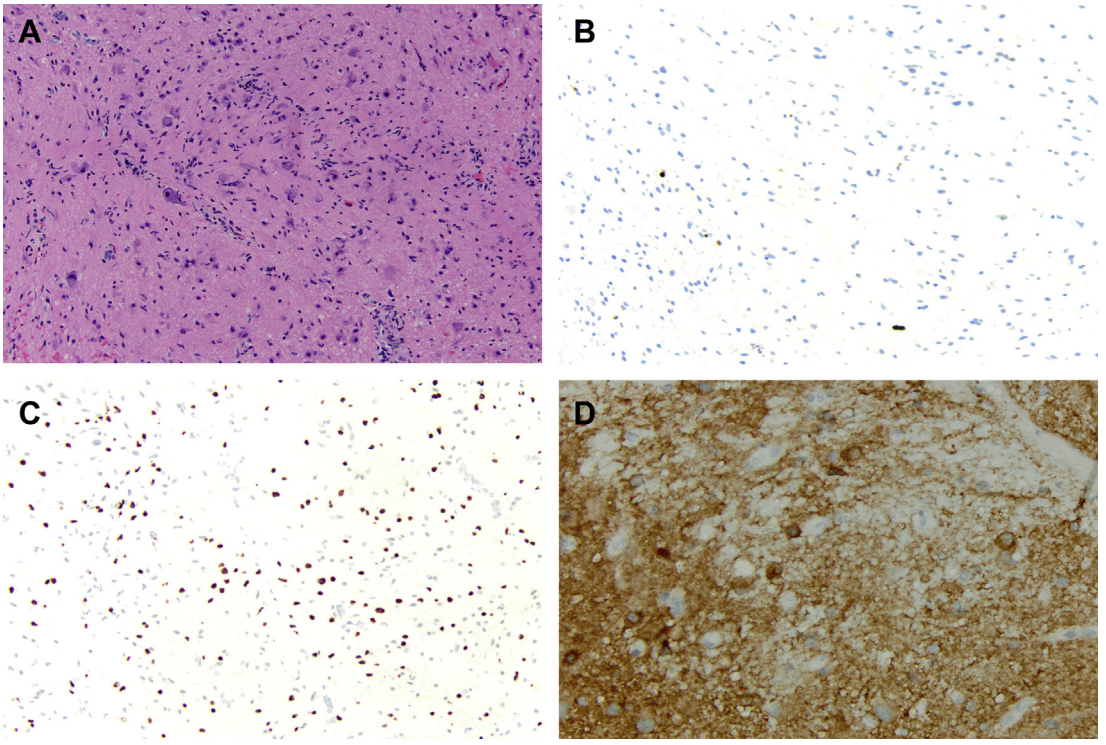

Fig. 9. A spinal ganglioglioma with *BRAF V600E* and *H3K27M* mutations. (*A*) Histologic examination showed a mildly cellular neoplasm composed of atypical glial cells admixed with clusters of large, disorganized neurons with dysplastic features (H&E). (*B*) The Ki67 immunostain showed a proliferation rate of approximately 1%. (*C*) The H3K27M immunostain was positive in the glial component and (*D*) BRAF V600E was positive in both glial and neuronal components.

similar midline pediatric gangliogliomas at WHO grade I (2 thalamic, 1 in the pons, 1 spinal, and 1 in the cerebellar penduncle) with co-occurring mutations in *BRAF V600E* and *H3K27M*. Of the 5 patients reported, 4 had follow-up, of which 3 were alive with stable disease at 9 months, 1 year, and 7 years, and 1 died of recurrent disease at 8 years. This series suggests that not all H3K27M-mutated gliomas should be graded as WHO IV. *H3K27M* mutations can also co-occur with *FGFR1* point mutations; however, the outcome implication of this association is not fully described.[53] Given the existent short series and case reports on such cases, the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy Now Working Committee 3 released clarifying diagnostic criteria suggesting that the diagnosis of diffuse midline glioma, H3K27M mutant, WHO grade IV should be reserved for those gliomas that are diffusely infiltrating, in a midline location, and harboring an *H3K27M* mutation.[54] Given that many PAs and gangliogliomas show areas of infiltration and can occur in midline locations, it is possible that these guidelines can still allow for challenges in diagnosis. In challenging diagnoses, analyzing the co-occurring genetic events of an *H3K27M*-mutant glioma is useful, because the WHO grade IV gliomas typically have alterations in *PDGFRA*, *ACVR1*, *ATRX*, and *TP53*.[45]

Bona fide diffuse midline gliomas with *H3K27M* mutations have a 2-year overall survival of less than 10%.[3] Factors associated with longer survival include younger age, longer symptom latency, and absent ring enhancement on MRI.[55] A large study of more than 1000 patients with radiographically confirmed DIPG showed additional prognostic information, including that *H3.1 K27M* is associated with longer survival than *H3.3 K27M*.[56]

Of practical significance, although the availability of a mutation-specific H3K27M antibody facilitates the diagnosis,[57] awareness of limitations should be observed. First, the H3K27M antibody detects mutations associated with more than just a single gene. In our experience, dark nuclear staining is more associated with *H3F3A* mutation, whereas lighter, more speckled staining pattern is associated with *HIST1H3B* mutation. Furthermore, staining should only be interpreted as positive when observed in tumor nuclei (not cytoplasmic expression) and in the context of appropriate positive and negative controls (see **Fig. 8**). In addition, although H3K27M-mutant tumors are associated with loss of H3K27me3 expression, the latter is not specific for *H3K27M* mutation and should not be used as a surrogate for the mutation-specific antibody.[58]

BRAF V600E MUTATION IN HIGH-GRADE GLIOMAS

The most common *BRAF* alteration encountered in high-grade gliomas is *BRAF V600E*. Mistry and colleagues,[59] in a study of 886 patients with pediatric low-grade glioma, analyzed 26 cases that progressed to secondary high-grade gliomas. The most common alteration in secondary high-grade gliomas was *BRAF V600E* and *CDKN2A* loss, and all high-grade gliomas harboring these alterations could be traced back to their low-grade counterparts, although the transformation was of long duration. High-grade gliomas with *BRAF V600E* mutation tended to also have *TP53* mutations. It was concluded that *BRAF V600E* mutations and *CDKN2A* deletions constitute a clinically distinct subtype of high-grade glioma. None of the high-grade gliomas in this cohort from Sick Kids Hospital had *KIAA1549-BRAF* fusion. Given the available targeted therapeutic options in clinical trials, it is important to determine whether a glioma harbors *BRAF* alterations and the type of alteration. Clinical trials usually include strata for *BRAF V600E*–mutant tumors and for without them, because *BRAF V600E* inhibitors can paradoxically activate the MAPK pathway through ERK signaling in *BRAF*-fused gliomas.[60] The BRAF V600E immunohistochemical stain is a good tool to screen for mutation; however, its interpretation can be uncertain and molecular confirmation through digital droplet polymerase chair reaction or a targeted sequencing panel that includes the *BRAF* gene is more sensitive.

NTRK REARRANGEMENTS IN HIGH-GRADE GLIOMAS

NTRK fusions can be seen in both low-grade and high-grade gliomas,[31,33] and their morphology is variable. In our clinical experience, it is difficult to assign a histologic grade to a subset of gliomas with *NTRK* rearrangement because their histology does not fit perfectly in any of the conventional entities. A case encountered in practice shows this (**Fig. 10**): a 3-year-old child presented with a brief history of headaches, and imaging showed a third ventricular well-defined tumor with an area of infiltration in the midbrain. A biopsy was performed and it showed a minimally infiltrative tumor composed of back-to-back monomorphic cells with round to oval nuclei and abundant eosinophilic cytoplasm. Occasional mitoses were seen, but there was no microvascular proliferation or necrosis. Eosinophilic granular bodies and Rosenthal fibers were not present. The tumor was diffusely positive for GFAP and

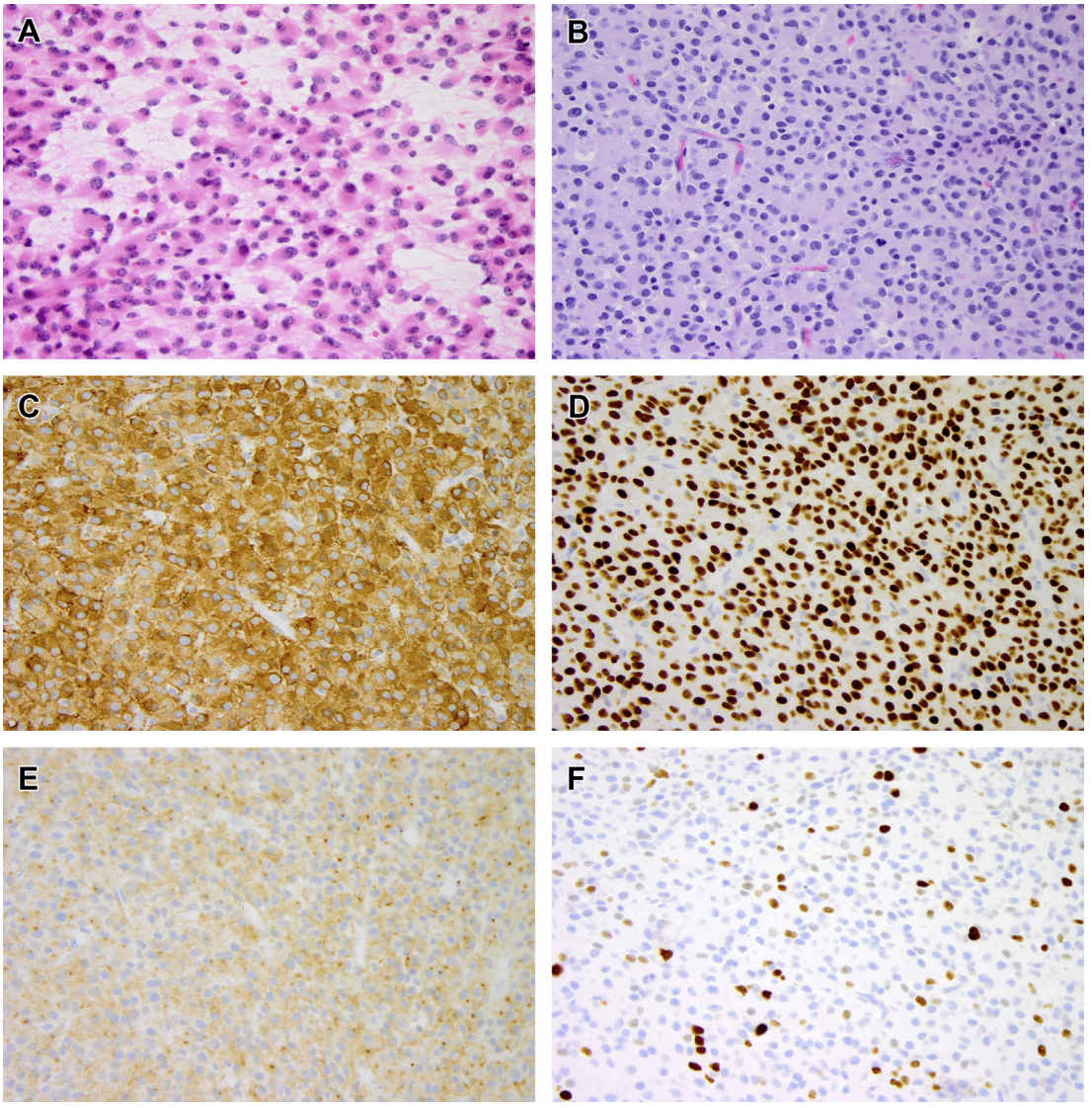

Fig. 10. An *NTRK3-EV6*–rearranged glioma of intermediate grade. (*A*) The intraoperative smear preparation showed a monomorphic population of cells with eccentric nuclei and abundant eosinophilic cytoplasm. Definitive processes were not seen (H&E). (*B*) The paraffin-embedded H&E section showed a tumor composed of monomorphic epithelioid cells with sheetlike pattern of growth as well as occasional papillaelike structures. (*C*) The GFAP and (*D*) OLIG2 immunostains were diffusely positive, in keeping with a glioma. (*E*) Synaptophysin was expressed in a cytoplasmic and dotlike pattern. (*F*) The Ki67 immunostain shows areas of moderately increased proliferation. There was no necrosis and no microvascular proliferation.

OLIG2 and also showed some patchy synaptophysin expression. The Ki67 was focally increased to 7%. Given the presence of occasional mitoses in a small biopsy, by WHO grading scheme, there was concern for a grade III tumor; however, the tumor appeared monomorphic and mostly noninfiltrative. Therefore, a diagnosis of glioma with some concerning features was rendered and the child was placed on low-grade glioma therapy. A fusion panel showed an *NTRK3-ETV6* fusion. More than a year after the diagnosis, the tumor grew approximately 3 mm, which is atypical for a high-grade glioma, and the patient was placed on targeted therapy.

High-grade gliomas with *NTRK* fusions have all the histologic characteristics of glioblastoma.

Given the new progress in targeted therapy, uncovering *NTRK* rearrangements in pediatric gliomas is important for patient care. Because TRK proteins are expressed in the normal brain, panTRK immunostain is not a good immunosurrogate for gliomas, hence fluorescence in situ hybridization

or a fusion panel is more appropriate whenever a *NTRK*-rearranged glioma is suspected.

NEW HIGH-GRADE GLIOMA ENTITIES

In 2016, Sturm and colleagues[60] performed comprehensive genomic and methylation characterization of 323 tumors diagnosed as primary CNS-PNETs at multiple collaborating institutions. The study showed that most of the tumors clustered with known entities, and there were 4 new entities with distinct histology and biology: CNS neuroblastoma with *FOXR2* activation (CNS NB-*FOXR2*), CNS Ewing sarcoma family tumor with *CIC* alteration (CNS EFT-*CIC*), CNS high-grade neuroepithelial tumor with *MN1* alteration (CNS HGNET-*MN1*), and CNS high-grade neuroepithelial tumor with *BCOR* alteration (CNS HGNET-*BCOR*). Of these, tumors from the CNS HGNET-*MN1* and CNS HGNET-*BCOR* entities expressed GFAP, whereas neuronal immunomarkers were positive only focally or were negative. Most tumors in the CNS HGNET-*MN1* category were well circumscribed and high grade, and contained a mixture of solid and pseudopapillary patterns, some resembling astroblastoma. Because most of the tumors histologically diagnosed as astroblastoma were in this category, it was considered unlikely that there is a true astroblastoma entity other than the *MN1*-altered tumors found by this study.

The CNS HGNET-*BCOR* consisted of relatively well-defined tumors with glial features composed of spindle and oval cells arranged in perivascular pseudorosettes. The *BCOR* alteration found in these tumors is a tandem duplication in exon 15, which is the same alteration described in clear cell sarcomas of kidney. **Fig. 11** shows the histology of such a case encountered in our clinical practice. Recently, Torre and colleagues[29] described 3 gliomas with *BCOR-EP300* fusion and distinct morphology that clustered together and separately from any other entities, including CNS HGNET-*BCOR*, on methylation clustering analysis, indicating that they represent a specific entity. This discovery also suggests that a more specific nomenclature for CNS HGNET-*BCOR* is needed. All 3 tumors in this study were large supratentorial gliomas with a myxoid/microcystic background stroma; frequent calcifications, including psammomatous; and prominent chicken-wire vessels. All 3 cases had areas of low-grade morphology and 2 of them also showed areas of anaplasia, potentially suggesting progression from a low-grade to a high-grade lesion. Representative photographs of such a case are shown in **Fig. 12**. BCOR immunohistochemical stain is a reliable surrogate for *BCOR* gene alterations in gliomas and it is positive in both HGNET-*BCOR* ITDex15 and in *BCOR-EP300* gliomas (see **Fig. 12**).

RADIATION-INDUCED HIGH-GRADE GLIOMAS

Among pediatric high-grade gliomas, the tumors that are secondary to prior radiotherapy should be mentioned. Radiotherapy improves survival of many pediatric cancers, but one of the side effects is increased risk of radiation-induced malignancy. Gliomas can arise after cranial or craniospinal radiation; almost all of them are high-grade and have particularly unfavorable outcome. In a series of 12 radiation-induced gliomas described by Lopez and colleagues,[61] 10 were high grade; of those, 7 harbored *TP53* mutations. The second most common genetic alteration was homozygous deletion of *CDKN2A* and *CDKN2B*. Other alterations encountered were *PDGFRA* and *MET* amplification, and *BRAF* and

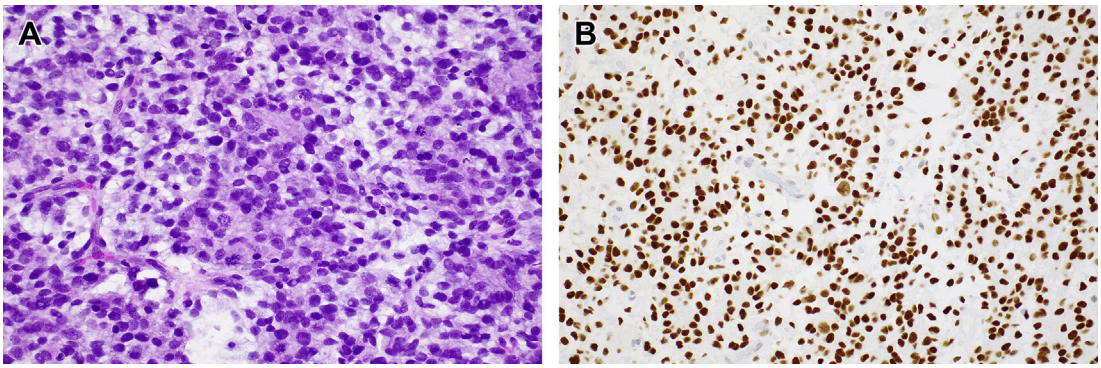

Fig. 11. HGNET-BCOR ITDex15 (*A*) Glioma composed of cells with round to oval atypical nuclei with vague perivascular arrangement around delicate vessels. Mitotic and apoptotic figures were easily found. (*B*) Nuclear BCOR immunostains were present in all tumor cells.

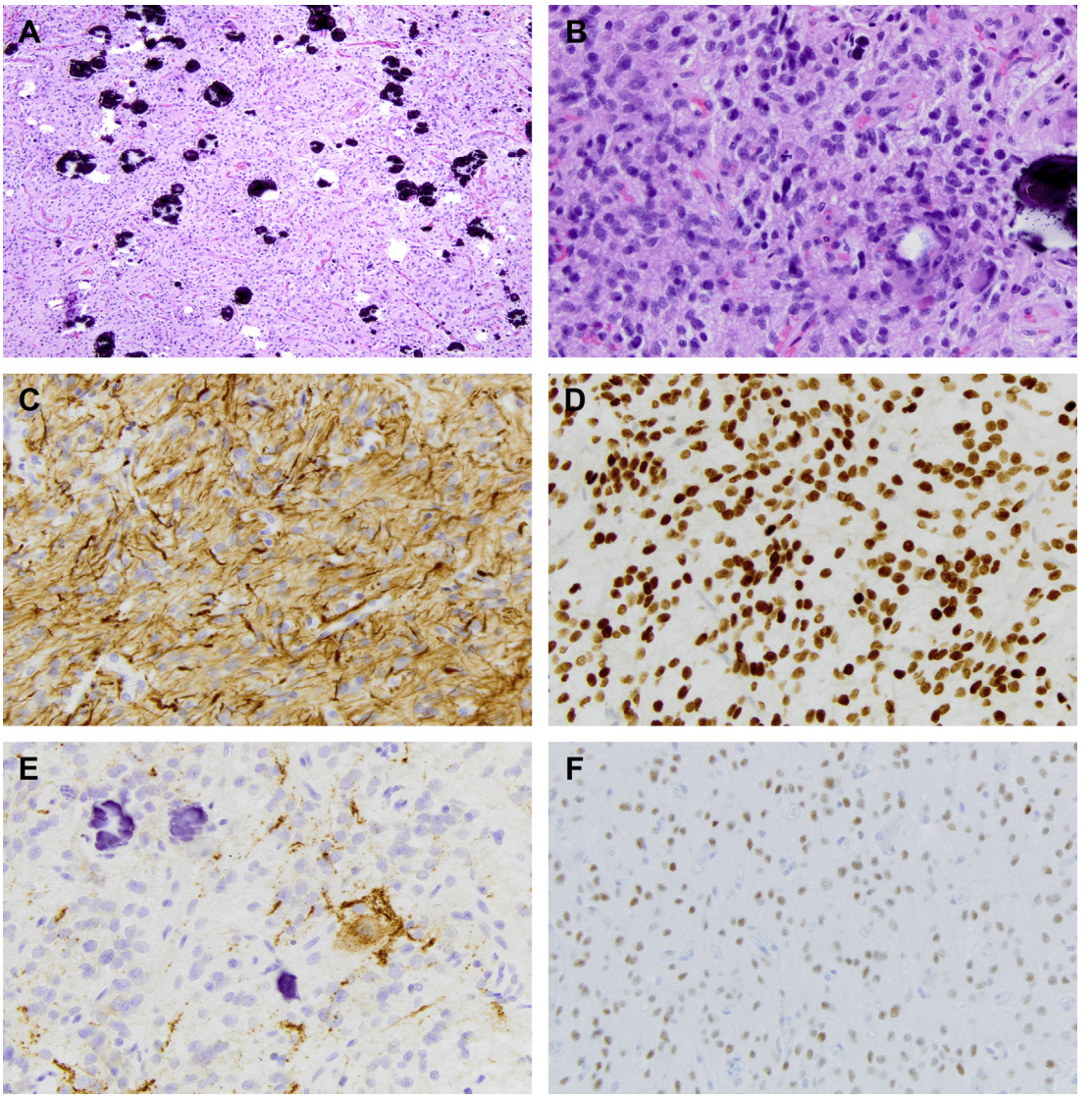

Fig. 12. Glioma with BCOR-EP300, showing (*A*) areas with low-grade histology with frequent psammomatous calcifications; moderate cellularity; mild atypia; and a network of thin, delicate vessels (H&E). (*B*) Areas of increased cellularity and occasional mitoses (H&E). GFAP (*C*) and OLIG2 (*D*) immunostains were diffusely positive and the tumor was negative for synaptophysin (*E*; highlights indicate entrapped axons and neurons). (*F*) A BCOR immunostain is positive in tumor nuclei.

RRAS2 mutations. All tumors lacked alterations in *IDH1*, *IDH2*, *H3F3A*, and *HIST1H3B/C*, as well as *TERT* and *PTEN*, and had low somatic mutation burden. Chromosomal copy number analysis of the high-grade gliomas in this cohort showed multiple chromosomal gains and losses per tumor, and their number was significantly higher than in spontaneous high-grade gliomas, suggesting that the copy number changes might happen somatically at the time of radiation.

PRACTICAL APPROACH TO THE DIAGNOSIS OF PEDIATRIC GLIOMAS

In a time when targeted therapy is available for a subset of pediatric gliomas and treatment options are evolving rapidly, the molecular features of a tumor are a pivotal component of a pathology report. Surgical specimens of gliomas are often limited and tissue triaging is the first important step in reaching a detailed but specific diagnosis. Tissue

conservation and test selection are important. Ordering unstained slides upfront with no waste, separating a fragment of tissue into 2 blocks even if it can be accommodated in 1, avoiding immunostains that do not bring the case closer to a specific diagnosis (eg, GFAP in a clearly glial neoplasm, IDH1 in an infant) are just a few of the techniques that can be used to conserve material. Molecular test selection depends on tumor type: many pediatric gliomas harbor fusions between genes on the same chromosome, meaning that either a comprehensive fusion panel or a gene-specific fluorescence in situ hybridization probe, such as *BRAF*, *FGFR*, the *TRK* family of genes, *MYB*, *ALK1*, or *ROS1*, will be needed. Some targeted exome sequencing panels contain algorithms that investigate for the presence of fusions in a selected number of genes.[35] Although immunosurrogate markers for molecular alterations are useful in reaching a diagnosis, most clinical trial enrollments and targeted therapies require molecular confirmation of immunohistochemical findings.

Given the limited standard therapeutic options, particularly in high-grade gliomas and infantile gliomas, an integrated pathologic diagnosis that includes the histologic and molecular findings is of utmost importance in guiding clinical management.

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