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High-Grade Astrocytoma With Piloid Features

Case Series and Review of a Recently Described Brain Tumor Entity

Mark A. Rudolf, MD, PhD; Sean P. Ferris, MD, PhD

• ***Context.***—**High-grade astrocytoma with piloid features (HGAP) is a newly recognized glioma defined by its methylation profile. Understanding of its clinical, histologic, and molecular characteristics continues to evolve.**

Objective.—**To review the HGAP literature, emphasizing updates in our understanding of the entity since its codification in the 2021 World Health Organization (WHO) Blue Book. Additionally, to present a case series illustrating a single institutional experience with HGAP.**

Data Sources.—**The English-language HGAP literature from 2018 to 2024 was reviewed. Four cases of HGAP were reviewed, along with relevant medical records.**

Conclusions.—**HGAP is an important consideration in the differential diagnosis of isocitrate dehydrogenase–wild-type gliomas and is more frequently encountered in adults. A handful of studies published following the entity's codification in the 2021 WHO Blue Book have refined our understanding of its clinical, histologic, and hallmark molecular characteristics. The most substantial updates include the description of 3 provisional subtypes, further characterization of an association with neurofibromatosis 1 syndrome, identification of new rare molecular alterations, and documentation of a unique case of possible transformation of pilocytic astrocytoma into HGAP. Clues to the diagnosis of HGAP include histologic infiltrating glioma with moderate pleomorphism, posterior fossa location, *CDKN2A/B* (cyclin dependent kinase inhibitor 2A/B) deletion, MAPK (mitogen-activated protein kinase) pathway alterations, ATRX (alpha thalassemia/mental retardation syndrome X-linked) loss, and association with neurofibromatosis 1 syndrome in some cases; these findings should prompt further molecular testing, including genome-wide DNA methylation analysis, which is currently essential for diagnosis.**

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Genome-wide DNA methylation analysis (methylation profiling) is a useful technology to "fingerprint" tumors based on their epigenetic state[1] and has been effectively leveraged as a classification tool for tumors of the central nervous system (CNS).[2] High-grade astrocytoma with piloid features (HGAP) is a newly described CNS tumor entity defined by its unique methylation profile, initially described when Reinhardt et al[3] examined a large cohort of histologically defined anaplastic pilocytic astrocytomas (PAs). Since HGAP was formally codified by the World Health Organization (WHO) "Blue Book" classification in 2021,[4] our understanding of the clinical, histologic, and molecular characteristics of HGAP has continued to evolve as larger and more diverse patient cohorts are assessed. Here, we provide an update on the HGAP literature and present a series of 4 HGAP cases.

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From the Department of Pathology, University of Michigan Medical School, Ann Arbor.

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Corresponding author: Sean P. Ferris, MD, PhD, Pathology Division of Neuropathology, University of Michigan Medical School, Department of Pathology, 2800 Plymouth Rd, Bldg 35, Ann Arbor, MI 48109 (email: spferris@med.umich.edu).

DATA SOURCES

For the review of HGAP literature, a PubMed search query of "anaplastic astrocytoma with piloid features" OR "high-grade astrocytoma with piloid features" on April 29, 2024, yielded 42 publications, 28 of which were published in 2018 or later, when the methylation class currently associated with HGAP was first described.[3] Of these, 12 publications presented HGAP cases supported by methylation data (see Table 1). In studies that provided case-level calibrated scores for methylation data, statistical values from cases with high-confidence methylation scores (cutoff $\geq$0.84) were compiled.[5] Both heterozygous and homozygous deletions of *CDKN2A/B* (cyclin dependent kinase inhibitor 2A/B) are included in summary statistics, consistent with prior studies[6] (Table 1).

The case series was approved by the University of Michigan Medical School Institutional Review Board (HUM00254975; Ann Arbor, Michigan) with waived patient consent owing to its retrospective nature. Medical and pathologic records were reviewed from 4 patients who were diagnosed with HGAP via high-confidence methylation studies at our institution. Patient 1 had been included in a prior published cohort,[6] and data from patients 2 through 4 have not been previously reported.

EPIDEMIOLOGY AND CLINICAL FEATURES

HGAP most commonly arises in the posterior fossa (62% of cases) and is less frequent in supratentorial (26%) or

Table 1. Summary of Studies Presenting Methylation-Supported Cases of High-Grade Astrocytoma with Piloid Features

Source, y	No. of HGAP Cases (High Confidence[a])	Cohort	*CDKN2A/B* Deletion, No. (%)	ATRX Loss/ Mutation, No. (%)	MAPK Alteration, No. (%)
Reinhardt et al, 2018[3]	83 (N/A)[b]	102 cases of histologically defined anaplastic PA	66/83, 80[b]	33/74 (45)[b]	49/67 (75)[b]
Reinhardt et al, 2019[11]	27 (25)[c]	86 cases of cerebellar GBM	20/25 (80)	6/25 (24)	13/25 (52)
Gareton et al, 2020[8]	1 (1)	31 pediatric cases of PA with anaplastic histology and MAPK pathway alteration	0/1 (0)	0/1 (0)	1/1 (100)
Bender et al, 2021[7]	6 (4)	951 cases of newly diagnosed glioma	4/4 (100)	4/4 (100)	1/4 (25)
Biczok et al, 2021[18]	4 (1)	26 cases of spinal astrocytoma	1/1 (100)	0/1 (0)	N/A
Lucas et al, 2022[10]	5 (4)	32 cases of glioma arising in patients with NF1 syndrome	4/4 (100)	4/4 (100)	4/4 (100)
Suruga et al, 2022[16]	1 (0)	4 elderly patients with tumors of PA morphology	N/A	N/A	N/A
Cimino et al, 2023[6]	144 (120)[d]	144 cases matching to methylation-class HGAP	106/120 (88)	60/98 (61)	87/120 (72.5)[b]
Picart et al, 2023[12]	3 (1)	83 patients with adult cerebellar GBM, with 17 tumors subject to methylation analysis	N/A	N/A	1/1 (100)
Kleinschmidt-DeMasters and Ormond, 2023[13]	2 (1)	10 cases of high-grade glioma with leptomeningeal metastasis or dural spread	1/1 (100)	1/1 (100)	1/1 (100)
Alturkustani, 2023[9]	1 (1)	19 cases of pediatric PXA	1/1 (100)	N/A	1/1 (100)
Soni et al, 2024[21]	8 (8)	8 cases with integrated diagnosis of HGAP	7/8 (87.5)	7/8 (87.5)	8/8 (100)
Current series	4 (4)	4 cases with integrated diagnosis of HGAP	3/3 (100)	4/4 (100)	1/4 (25)
Total	**172 (140)**		**122/138** (88)	**75/116** (65)	**117/168** (70)[b]

Abbreviations: ATRX, alpha thalassemia/mental retardation syndrome X-linked; *CDKN2A/B*, cyclin dependent kinase inhibitor 2A/B; GBM, glioblastoma; HGAP, high-grade astrocytoma with piloid features; MAPK, mitogen-activated protein kinase; N/A, not applicable; NF1, neurofibromatosis 1; PA, pilocytic astrocytoma; PXA, pleomorphic xanthoastrocytoma.

[a] Methylation score $\geq$0.84.
[b] No methylation score reported/includes cases with score $<$0.84.
[c] Includes cases from Reinhardt et al[3] (2018).
[d] Includes cases from Reinhardt et al[3] (2018), Bender et al[7] (2021), and patient 1. Four cases with high-confidence match to HGAP on 1 classifier version and high-confidence match to GBM on another version were not included in the high-confidence total.

spinal (10%) regions.[6] The tumor entity is uncommon, with one institution documenting 6 cases of HGAP in a 3-year span during which 951 patients were treated for newly diagnosed glioma.[7] The median age at diagnosis is 43 years,[6] and despite a reported range of 4 to 88 years,[6] HGAP is particularly rare in the pediatric population; just 9% of HGAP cases have occurred in patients younger than 18 years,[6] and in one study of pediatric cases of pilocytic astrocytoma with anaplastic features, only 1 of 31 tumors matched to HGAP by methylation studies.[8] Rarely, it appears that HGAP may arise via transformation of pilocytic astrocytoma. Indeed, 1 unique case has been reported of a 20-year-old whose low-grade basal ganglia tumor matched to pilocytic astrocytoma via methylation profiling on initial resection before recurring 6 years later as a high-grade tumor matching to HGAP by methylation profling.[9] However, evidence for this was not identified in the largest published cohort of HGAP cases, with 3 patients additionally showing paired initial and recurrent tumors matching to HGAP by methylation profiling.[6]

Although rare, HGAP cases are enriched in specific clinical scenarios. Approximately 9% of cases occur in patients with neurofibromatosis type 1 (NF1) syndrome.[6] In one cohort of patients with NF1 syndrome, HGAP comprised 5 of 32 gliomas (16%) that underwent methylation profiling,[10] the proportion rising to 5 of 14 (36%) when considering the subset of those tumors with high-grade molecular features.[10] Tumors previously diagnosed as "cerebellar glioblastoma" also appear to be enriched for HGAP. One comprehensive molecular analysis of 86 such tumors found that approximately one-third could be reclassified as HGAP upon methylation profiling.[11] Another report found that 3 of 17 cerebellar glioblastomas (18%) diagnosed from 2003 to 2017 could be reclassified as HGAP, although only a fraction of tumors in that cohort underwent methylation testing.[12]

Prognostic data are somewhat limited. An initial study demonstrated a 5-year overall survival rate of 50%,[3] although long-term survival greater than 25 years has also been reported.[6] Cimino et al[6] identified an epigenetic subtype of HGAP enriched for patients with NF1 syndrome (gNF1), which appeared to be confined to the posterior fossa and was associated with poorer progression-free survival. One case series of metastatic gliomas reported that 2 HGAP tumors exhibited leptomeningeal spread, showing the potential for aggressive behavior in this entity.[13] A definitive CNS WHO grade is currently not indicated, but at the time of publication of the 2021 CNS WHO, clinical behavior was thought to roughly correspond to CNS WHO grade 3.

HISTOPATHOLOGY

The histologic features of HGAP are varied. Most HGAPs appear to be moderately pleomorphic, infiltrative astrocytic gliomas (often resembling glioblastoma or pleomorphic xanthoastrocytoma), and a subset show a predominance of hairlike (piloid) processes, mimicking pilocytic astrocytoma or "anaplastic" pilocytic astrocytoma.[3] In an expanded cohort of 144 patients with HGAP, researchers questioned whether the "piloid" designation is warranted, as most tumors in their study demonstrated glioblastoma-like histology, rather than pilocytic astrocytoma-like histology.[6] Rosenthal fibers or eosinophilic granular bodies were present in approximately one-third of cases, and approximately two-thirds showed microvascular proliferation and/or necrosis.[6] Around 80% of tumors show 1 or more mitoses per 10 high-power fields,[3] and the Ki-67 index ranges from 1% to 30%.[6] Immunohistochemical staining is helpful, as HGAP tumors are isocitrate dehydrogenase (IDH)–wild type and overwhelmingly negative for H3 K27M immunostaining[3]; furthermore, ATRX (alpha thalassemia/mental retardation syndrome X-linked) loss/mutation occurs in approximately 44% to 60% of HGAPs.[3,6] Recent methylation analyses suggest that the cellular composition of the tumor microenvironment differs among 3 different epigenetic subtypes of HGAP.[6]

MOLECULAR FEATURES

Genome-wide DNA methylation studies are currently an essential diagnostic feature of HGAP,[4] and 140 of 172 reported cases (81%) matched with a calibrated score of 0.84 and above (Table 1). Tumors are exclusively IDH–wild type, and the presence of H3 K27M mutation or *EGFR* (epidermal growth factor receptor) amplification is considered unusual.[3,4,6] In the largest published cohort of HGAP cases, the most common genetic alterations include *CDKN2A/B* deletion (84%), ATRX loss/mutation (59%), MAPK (mitogen-activated protein kinase) pathway alterations (72.5%), and *CDK4* (cyclin dependent kinase 4) amplification (12%).[6] Similar rates are calculated when compiling all published HGAP cases with high-confidence methylation scores (Table 1). The most common MAPK pathway alterations include *NF1* alterations (40.4%), *FGFR1* (fibroblast growth factor receptor 1) mutation or fusion (19% and 14%, respectively), and *BRAF* (B-Raf proto-oncogene, serine/threonine kinase) fusion or V600E mutation (19% and 2%, respectively).[6] Rare mutations in *TP53* (tumor protein p53; 5%) and *NTRK* (neurotrophic receptor tyrosine kinase; single case) have been newly described.[6]

Most HGAPs show 4 or more structural aberrations on copy number profile analysis (88%), particularly 9p deletions (*CDKN2A/B*) and fusions at 7q (*KIAA1549*::*BRAF*), as well as alterations of 12q and 17q, and deletions of 1p, 8p, and 19q.[3] A considerable percentage of HGAPs (45%) show MGMT (O-6-methylguanine-DNA methyltransferase) promoter hypermethylation,[3] though an association with response to alkylating chemotherapy is currently unknown.

Some molecular differences have been identified among the recently described epigenetic subtypes of HGAP (gNF1, g1, and g2).[6] The gNF1 group was found to have a greater incidence of germline *NF1* alterations, and ATRX loss/mutation was even more frequent in gNF1 (88%) than in g1 and g2 subtypes (60% and 48%, respectively).[6] The gNF1 group also was found to have differential methylation at various sites, including at NF1 enhancer regions and within RNA-processing pathways.[6]

DIFFERENTIAL DIAGNOSIS AND PRACTICAL DIAGNOSTIC APPROACH

Based on histology alone, the differential diagnosis of HGAP is broad and includes entities with CNS WHO grades 1 through 4 (pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and both low- and high-grade infiltrating astrocytomas). Presence of an IDH mutation would indicate an IDH-mutant glioma and excludes the diagnosis of HGAP. Very few HGAP cases show *TERT* (telomerase reverse transcriptase) promoter mutation and *EGFR* amplification, but the presence of these alterations in an IDH–wild-type infiltrating astrocytoma could erroneously suggest a diagnosis of IDH–wild type glioblastoma.[3,6] Considering the propensity of HGAP to involve midline structures, H3 K27M mutations (most commonly found in diffuse midline glioma, H3 K27-altered CNS WHO grade 4) have been reported in only 2 cases,[3] with diagnostic ramifications considered unclear.[4]

Pleomorphic xanthoastrocytoma and pilocytic astrocytoma can bear morphologic and molecular similarity to HGAP and may be impossible to distinguish histologically. Pleomorphic xanthoastrocytomas exhibit even higher rates of *CDKN2A/B* deletion (94%), but generally lack ATRX loss and harbor *BRAF* V600E mutation (76%),[14] whereas HGAP more commonly harbors *BRAF* gene fusion or alterations in *NF1* and *FGFR1*. PAs—the most common childhood glioma—also typically occur in the posterior fossa, and approximately two-thirds harbor *KIAA1549*::*BRAF* gene fusions.[15] Alterations in *NF1* and *FGFR1* are slightly less common in PA than in HGAP.[4] As mentioned above, HGAP is quite rare in the pediatric population, even in a cohort of PAs with histologic anaplasia.[8] Conversely, in cohorts of adult or elderly patients, tumors that resemble PA with histologic anaplasia are frequently reclassified as HGAP upon methylation profiling.[3,16] Thus, genome-wide DNA methylation analysis may be an important adjunct test for such cases.

When interpreting methylation results, scores of 0.84 or higher (in some cases $\geq$0.9) are considered a high-confidence match.[5,17] Generally, samples with low tumor cellularity have increased risk of failing to match.[17] Our review of the published HGAP literature finds that approximately 1 in 5 reported HGAP diagnoses was made in the absence of a high-confidence match on methylation profiling (Table 1). In these cases, tumors typically best matched HGAP on methylation profiling with a lower calibrated score, clustered with HGAP on dimensional reduction plots of methylome data, and held a constellation of other molecular characteristics such as ATRX loss or *CDKN2A*/B deletion.[6,7,10–13,16,18]

The fact that diagnosing HGAP currently requires methylation profiling and algorithmic classification raises several practical challenges and considerations. First, a sufficient number of HGAPs must be present within the reference cohort of a particular classifier for a case of interest to be recognized as HGAP. Both the German Cancer Research Center and National Institutes of Health/National Cancer Institute classifiers have this capability, but other classifiers with insufficient reference cases of HGAP may not. Second, methylation differences between sporadic and tumor predisposition syndrome–associated tumors have not been a primary consideration of initial methylation profiling studies. However, in one initial methylation profiling study,

Table 2. Summary of 4 Cases of High-Grade Astrocytoma With Piloid Features				
	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**
Clinical characteristics				
Age, y/Sex	81, male	57, male	42, male	33, female
NF1	No	Yes	No	Yes
Tumor site	Cerebellar vermis	Spinal cord	Ventral pons	Right superior midbrain/ posterior thalamus
OS	Alive 28 mo after surgery	Deceased 3 mo (sepsis)	Deceased 8 mo	Alive 1 mo after biopsy
Histology				
Morphology	Long glial processes on intraoperative smears—eosinophilic cytoplasm, round to ovoid nuclei with coarse punctate chromatin and scattered pleomorphism	Infiltrative astrocytic neoplasm with modest nuclear pleomorphism	Infiltrative glial cells with hyperchromatic nuclei	Infiltrating glioma with hyperchromatic, angulated, and occasionally pleomorphic nuclei within a fibrillary and myxoid background
Necrosis	No	No	Yes	No
MVP	Focal	No	No	No
Ki-67 index, %	3–8	7	N/A	12
ATRX IHC	Loss	Loss	Loss	Loss
Molecular studies				
CDKN2A/B	Heterozygous deletion	Heterozygous deletion	N/A	Homozygous deletion
MAPK alteration	*FGFR1* K656E mutation (p.G1312fs*2)	NF1 syndrome	None detected	NF1 syndrome
*MGMT*p	No hypermethylation	Hypermethylated	No hypermethylation	N/A
Methylation	High-confidence match HGAP (v12 NIH CNS classifier)	High-confidence match HGAP (v12 NIH CNS classifier)	0.93 HGAP (DKZF v11b4), 0.94 HGAP (DKZF v12.5)	0.99 HGAP (DKZF v12.8)

Abbreviations: ATRX, alpha thalassemia/mental retardation syndrome X-linked; *CDKN2A/B*, cyclin dependent kinase inhibitor 2A/B; CNS, central nervous system; DKFZ, Deutsches Krebsforschungszentrum (German Cancer Research Center); *FGFR1*, fibroblast growth factor receptor 1; HGAP, high-grade astrocytoma with piloid features; IHC, immunohistochemistry; Ki-67, marker of proliferation Ki-67; MAPK, mitogen-activated protein kinase; *MGMT*p, O-6-methylguanine-DNA methyltransferase promoter; MVP, microvascular proliferation; N/A, not applicable; NF1, neurofibromatosis 1; NIH, National Institutes of Health; OS, overall survival; v, version.

Capper et al[5] did note an "overproportional fraction" of unclassifiable tumors of unclear histologic diagnosis to be associated with hereditary tumor syndromes. Also, and as discussed above, continued research is beginning to identify associations between methylation subtypes and sporadic versus tumor predisposition syndrome–associated tumors.[6] Third, DNA methylation array–based classifiers require relatively pure tumor samples, and even state-of-the-art methods decline in performance when tumor cellularity is below 50%[19]; thus, it may be impossible to attain a high-confidence match to HGAP on specimens with low tumor cellularity. Finally, resources to perform methylation profiling may not be widely available. Emerging deep-learning analysis of histologic slides may provide a low-cost method to simulate DNA methylation profiling when HGAP is in the differential diagnosis.[20]

FOUR HGAP CASES

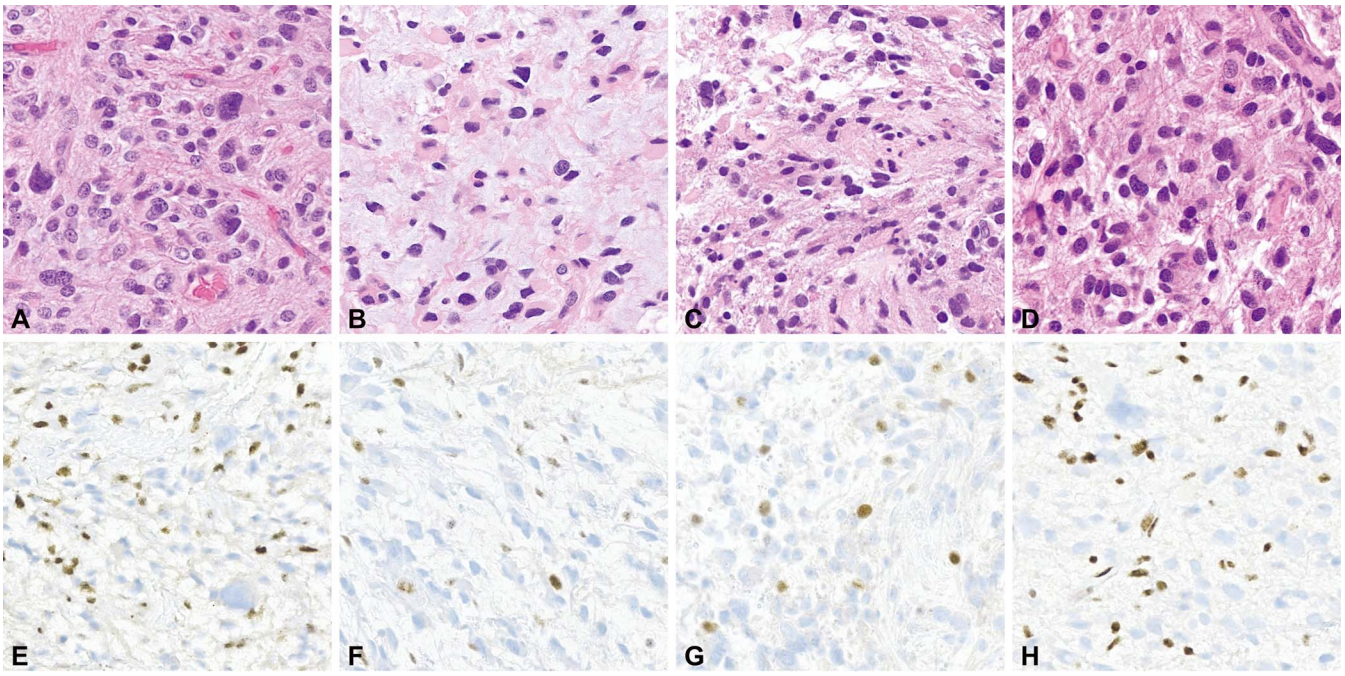
Four diagnoses of HGAP were rendered at our institution, each with a high-confidence match to HGAP via methylation analysis (see Table 2). All patients were adults (median age, 49.5 years; range, 33–81 years), and patients 2 and 4 presented with new lesions in the setting of NF1 syndrome. Presenting circumstances were unavailable for patient 3; in the other patients, tumors evoked neurologic signs and symptoms that prompted imaging.

The tumors in patients 1 to 4 were centered in the cerebellar vermis, cervical spinal cord, ventral pons, and superior midbrain/thalamus, respectively. Magnetic resonance imaging showed at least focal enhancement in all cases.

Representative histology is shown in the Figure. All cases demonstrated infiltrative gliomas with at least focal pleomorphism (Figure, A through D). The tumor from patient 1 exhibited focal microvascular proliferation, the tumor from patient 3 exhibited necrosis, and tumors from patients 2 and 4 lacked either of these high-grade features. No Rosenthal fibers or eosinophilic granular bodies were identified among the 4 tumors. Immunohistochemical stains showed that all 4 tumors expressed glial fibrillary acidic protein (GFAP), lacked IDH1-R132H immunoreactivity, and had loss of ATRX immunostaining (Figure, E through H). By immunomorphology, the differential diagnosis for all cases included HGAP, high-grade IDH-mutant glioma, and IDH–wild type glioblastoma.

Next-generation sequencing (NGS) using an in-house OncoMine Focus Assay (Thermo Fisher Scientific) solid tumor panel confirmed the absence of mutations in *IDH1* and *IDH2* in all cases. No *BRAF* alterations were identified, and aside from an activating *PIK3CA* p.E545K mutation and *CDK4* amplification in the tumor from patient 1, no other genetic alterations were identified. Of note, the *NF1* gene is not included in this solid tumor NGS panel. Further NGS testing of the tumor from patient 1, using the TruSight Oncology 500 panel (Illumina), demonstrated additional mutations, including *FGFR1* p.K656E, a MAPK-activating mutation.

Testing with the OncoScan CNV Plus microarray (Thermo Fisher Scientific) revealed heterozygous deletion



Histologic images of 4 tumors diagnosed as high-grade astrocytoma with piloid features (patients 1–4, from left to right). Hematoxylin-eosin staining demonstrated at least focal moderate pleomorphism in each tumor (A through D). All tumors showed loss of ATRX (alpha thalassemia/mental retardation syndrome X-linked) immunostaining in tumor cells (E through H), a finding reported in approximately 44% to 60% of cases[3,6] (original magnification ×400 [A through H]).

of *CDKN2A/B* in tumors from patients 1 and 2 as well as homozygous deletion of *CDKN2A/B* in the tumor from patient 4. (Although HGAP cases tend to harbor homozygous deletions of *CDKN2A/B*, at least 3 other cases with heterozygous loss have been reported.[7,11]) The tumor from patient 3 was not submitted for chromosomal microarray analysis owing to scarcity of tissue. None of the tumors exhibited concurrent gain of chromosome 7 and loss of chromosome 10. Methylation profiling demonstrated a high-confidence match to HGAP in all 4 cases, securing the diagnoses.

Patient 1 had received neoadjuvant temozolomide and radiation—both of which were discontinued owing to side effects—and was alive at last follow-up 28 months after subtotal resection. The postoperative course of patient 2 was complicated by sepsis with an overall survival of 3 months after subtotal resection. Patient 3 had an overall survival of 8 months after biopsy. Patient 4 was alive at last follow-up 1 month after biopsy.

CONCLUSIONS

Although the age range and location at presentation can vary widely, posterior fossa location, findings of an *IDH*–wild type astrocytic glioma with moderate pleomorphism, ATRX loss, *CDKN2A/B* deletion, and MAPK pathway alterations are clues to the diagnosis of HGAP and should prompt further molecular testing, including genome-wide DNA methylation studies that currently are required for definitive diagnosis. There also appears to be an increased incidence of HGAP in patients with NF1 syndrome. Since its codification in the 2021 WHO Blue Book, only a handful of methylation-supported HGAP studies have been published, with most cases presented within a lone large cohort.[6] That report describes 3 epigenetic HGAP subtypes and shows that namesake "piloid" features are only present in a minority of HGAP cases.[6] Further studies are required to determine the most effective therapeutic modalities and to better understand biologic, histologic, and prognostic differences among the epigenetic subtypes. Emerging computational methods may facilitate diagnostic workup when HGAP is in the differential diagnosis.[20] Continued recognition and diagnosis of HGAP will increase our understanding and advance therapies for this recently described glioma entity.

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