Importance of molecular testing in stratification of paediatric low-grade gliomas: a case study

Clare McGenity

Oleg Anichtchik

Azzam Ismail

This Short Case is brought to you in association with the Pathological Society of Great Britain and Ireland. Each month we feature a Short Case written by a member of the Trainees' Subcommittee of the Pathological Society of Great Britain and Ireland. This case, including its scanned slides, is published on the Pathological Society's website www.pathsoc.org/index.php/trainees/case-of-the-month (previous cases can be accessed by members via the education portal login). The Short Case includes a series of Test Yourself Questions at the end to check your understanding of the case. We hope you enjoy reading it.

Pathological Society
Understanding Disease — Guiding Therapy

Abstract

We discuss a case of a paediatric low grade glioma where the integrated diagnosis was a diffuse astrocytoma with MYB or MYBL1 alteration. Surgical excision of the tissue revealed a low grade tumour appearance with small round nuclei and fine fibrillary cytoplasm. MYB or MYBL1-alteration was shown on methylation array analysis. MYB and MYBL1 are transcriptional transactivators that have important roles in cell proliferation, and their overexpression can lead to tumour formation. The diagnosis of novel subtypes of paediatric low-grade gliomas is now possible due to the increasing role of molecular pathology.

Keywords Diffuse astrocytoma; molecular pathology; neuropathology; novel tumour; paediatric tumour

Case report

A 12-year-old male patient presented with a 2-day history of blurred vision and a background of six months of headaches, reported to worsen on bending forward. Associated vomiting was noted over the two weeks prior to hospital presentation. On examination, the patient had double vision on horizontal gaze and was noted to be clumsy on standing. Cranial nerves and bilateral power of the upper and lower limbs were within normal range. On CT scan, a 6 cm well circumscribed mass was identified and was obstructing the fourth ventricle. The lateral and third ventricles were dilated.

The patient was taken to theatre and underwent a posterior fossa craniotomy and debulking of the fourth ventricular brain stem tumour. Initial brain smears showed a low grade-glioma, thought to most likely represent a pilocytic astrocytoma (Figure 1). Further histology showed low-cellular patternless sheets of glioma, with cells containing small round nuclei, fine chromatin and fine fibrillary cytoplasm (Figures 2 and 3). There was no vascular proliferation or necrosis. The tumour showed a patchy infiltrative pattern on NFP staining (Figure 4). Immunohistochemistry for IDH1, p53 and BRAFV600E were negative and ATRX was nuclear retained (wild type) (Figure 5). Ki67 showed less than 1% proliferation. Based on these findings, histology confirmed intraoperative smear impression of a low grade glioma, with a pilocytic astrocytoma being among top differential diagnoses.

Samples were sent for molecular testing. Methylome EPIC array analysis was performed and showed a methylation class diffuse astrocytoma, MYB/MYBL1 altered subtype D (novel) with a score of 0.95. No BRAF, IDH1/2, TERT promoter or histone mutations or KIAA1549:BRAF gene fusion were identified with FISH and Next Generation Sequencing (NGS). MGMT promoter was unmethylated. Additional immunohistochemistry for BRAF V600E was negative, indicating no mutation.

The combined data from molecular pathology and histology allowed confirmation of the final integrated diagnosis of a diffuse astrocytoma, MYB or MYBL1-Altered, WHO grade 1.

Discussion

This case highlights the importance of comprehensive molecular pathology testing of paediatric tumours, as histological examination alone is no longer sufficient for diagnosis. The 2021 WHO classification of Tumours of the Central Nervous System recognises several novel tumour types, incorporating recent advances in identification of novel tumour-associated genetic alterations and emphasising distinction between adult-type and paediatric gliomas.[1]

Paediatric diffuse low-grade gliomas are a distinct group of brain tumours, generally associated with favourable outcomes.[2] This group comprises: diffuse astrocytoma with MYB or MYBL1 alteration; low-grade polymorphic juvenile neuroepithelial tumour; angiocentric glioma; diffuse low-grade glioma with MAPK pathway alteration.[2] Molecular techniques are crucial in distinguishing subtypes of this group of low-grade gliomas and can provide the patient and clinician with additional information about their likely disease course and treatment response.[3]

Diffuse astrocytoma with MYB or MYBL1 alteration are rare tumours of unknown aetiology and most often present as a

__Clare McGenity__ MBBS Digital Pathology Fellow, Leeds Teaching Hospitals NHS Trust, St James's University Hospital and Pathology and Data Analytics, University of Leeds, Leeds, UK. Conflicts of interest: none declared.

__Oleg Anichtchik__ MD PhD FHEA Specialty Registrar in Diagnostic Neuropathology, Leeds Teaching Hospitals NHS Trust, St James's University Hospital and Pathology and Data Analytics, University of Leeds, Leeds, UK. Conflicts of interest: none declared.

__Azzam Ismail__ BM BCH FRCPath Consultant Neuropathologist, Leeds Teaching Hospitals NHS Trust, St James's University Hospital, Leeds, UK. Conflicts of interest: none declared.

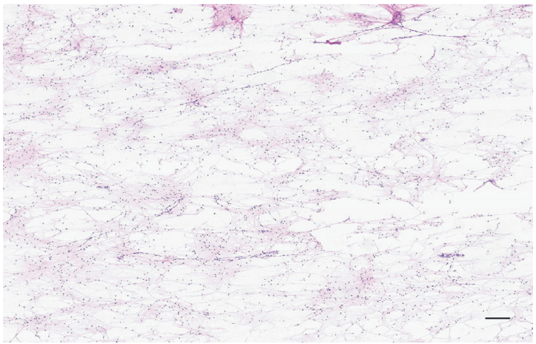

Figure 1 Brain smear with low to moderate cellularity demonstrating a low grade glioma at 5× magnification (200 μm scale).

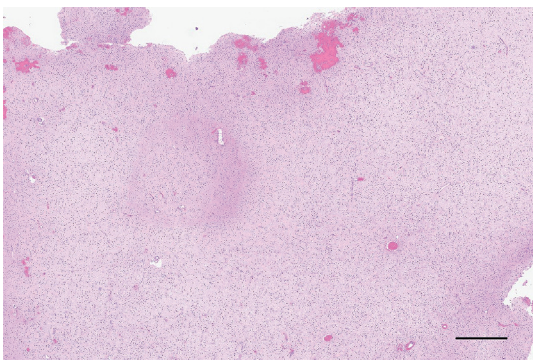

Figure 2 Posterior fossa low grade brain tumour appearances on H&E at 2.5× magnification (500 μm scale).

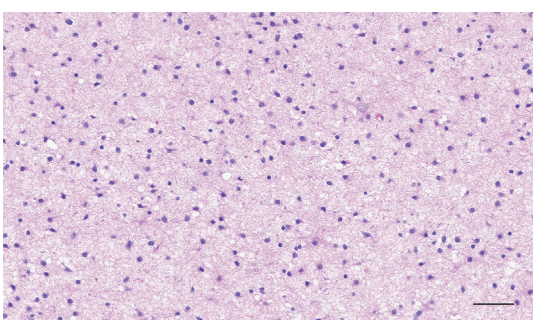

Figure 3 Posterior fossa low grade brain tumour appearances on H&E at 40× magnification demonstrating small round nuclei and fine fibrillary cytoplasm (50 μm scale).

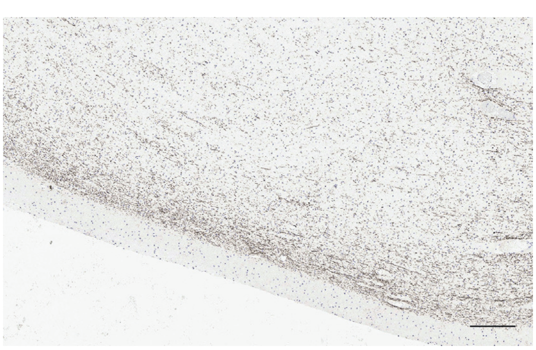

Figure 4 NFP shows positive fibres going through the tumour, suggesting a patchy infiltrative growth pattern of the tumour. 10× magnification (200 μm scale).

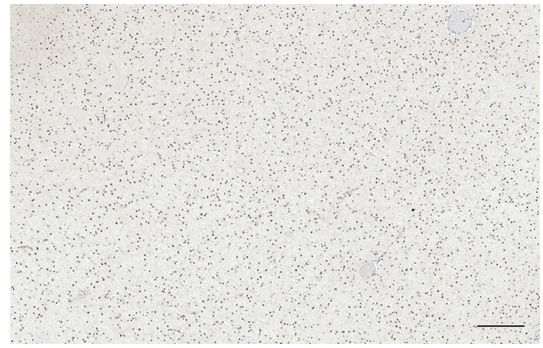

Figure 5 ATRX retained tumour at 10× magnification (200 μm scale).

cerebral neoplasm.[3] Whilst it was not the case with this patient's history, there is frequently a background of associated epileptic seizures in these patients.[3,4] As such, these tumours also fit into the broader group of "long-term epilepsy-associated tumours".[5] A good prognosis has been reported in this subtype of neoplasm, with a study by Wefers et al. (2020) recording no recurrence in patients following initial surgery and no additional radiotherapy or chemotherapy required within up to nine years of follow up.[5] 90% of patients become seizure free after resection.[5]

On imaging, diffuse astrocytoma with MYB or MYBL1 alteration is usually well defined but may sometimes show large cysts and some focal areas of a diffuse growth pattern.[5] They are thought to occur more commonly in male patients.[5]

Histology usually demonstrates monomorphic glial cells with bland, round nuclei and a fibrillar matrix.[3,6] These tumours are typically negative for GFAP and IDH1, have a low Ki-67 proliferation index and ATRX expression is retained.[3] There is some overlapping morphology with angiocentric gliomas and so these would be the most likely differential diagnosis to this tumour.[3]

MYB and MYBL1 are transcriptional transactivators that have important roles in cell proliferation.[7] Genetic alterations for these proteins can lead to overexpression resulting in gliomas and other cancers.[6] Sequencing, FISH and other molecular techniques can indicate this tumour subtype and are essential for reaching the diagnosis.[3]

As per the WHO 2021 diagnostic criteria, diffuse astrocytoma, MYB or MYBL1 altered must have the appearances of a diffuse astrocystoma without histological features of anaplasia. It must have no mutations in IDH or H3 genes. Additionally, it must either have a structural variant of MYB or MYBL1; or the DNA methylation profile must be in keeping with this tumour type.

Conclusion

Molecular testing is essential in the diagnosis of many brain tumours and is responsible for determining novel subtypes, where prognosis and treatment may vary when compared to broader conventional diagnostic groups. Novel brain tumour subtypes are emerging with advancement of these techniques, allowing progression to more personalised care for patients. ◆

REFERENCES

1 Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous System: a summary. *Neuro Oncol* 2021; **23:** 1231–51.

2 Fabbri VP, Caporalini C, Asioli S, et al. Paediatric-type diffuse low-grade gliomas: a clinically and biologically distinct group of tumours with a favourable outcome. *Pathologica* 2022; **114:** 410.

3 WHO Classification of Tumours Editorial Board. World health organization classification of tumours of the central nervous system. Lyon: IARC Press, 2021.

4 Slegers RJ, Blumcke I. Low-grade developmental and epilepsy associated brain tumors: a critical update 2020. *Acta neuropathologica communications* 2020; **8:** 1–11.

5 Wefers AK, Stichel D, Schrimpf D, et al. Isomorphic diffuse glioma is a morphologically and molecularly distinct tumour entity with recurrent gene fusions of MYBL1 or MYB and a benign disease course. *Acta neuropathologica* 2020; **139:** 193–209.

6 Qaddoumi I, Orisme W, Wen J, et al. Genetic alterations in uncommon low-grade neuroepithelial tumors: BRAF, FGFR1, and MYB mutations occur at high frequency and align with morphology. *Acta neuropathologica* 2016; **131:** 833–45.

7 Brayer KJ, Frerich CA, Kang H, et al. Recurrent fusions in MYB and MYBL1 define a common, transcription factor–driven oncogenic pathway in salivary gland adenoid cystic carcinoma. *Cancer Discov* 2016; **6:** 176–87.

Practice points

- Paediatric diffuse low-grade gliomas are rare tumours and generally associated with favourable outcomes
- Diffuse astrocytoma with MYB or MBL1 alteration has a known association with childhood epilepsy and is one of a group of long-term epilepsy-associated tumours
- Molecular profiling is essential in determining subtypes of many brain tumours
- Diffuse astrocytoma with MYB or MYBL1 alteration is a novel subtype of paediatric low grade glioma that relies on molecular techniques for diagnosis

Self-assessment multiple-choice questions

1. Which of these is NOT a subtype of paediatric diffuse low-grade glioma?

a. Low-grade polymorphic juvenile neuroepithelial tumour
b. Diffuse astrocytoma with MYB or MYBL1 alteration
c. Infant-type hemispheric glioma
d. Angiocentric glioma

2. What condition is most associated with diffuse astrocytoma with a MYB or MYBL1 alteration?

a. Cerebrovascular accident
b. Epilepsy
c. Alzheimer's disease
d. Parkinson's disease

3. What type of test is used to assess methylome profile of these brain tumours?

a. Immunohistochemistry
b. FISH
c. Next generation sequencing
d. EPIC array

Answers

1 c
2 b
3 d