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RESEARCH ARTICLE

Brain Pathology

Molecular profile of adult primary leptomeningeal gliomatosis aligns with glioblastoma, IDH-wildtype

Yi Zhu[1] | **Darin D. Carabenciov**[2] | **Derek R. Johnson**[3] | **Jorge A. Trejo-Lopez**[1] | **Aivi T. Nguyen**[1] | **Aditya Raghunathan**[1] | **Giuseppe Lanzino**[4] | **Cristiane M. Ida**[1] | **Cinthya J. Zepeda-Mendoza**[1] | **Surendra Dasari**[1] | **Emilie Russler-Germain**[5] | **Sonika Dahiya**[5] | **Martha Quezado**[6] | **Kenneth Aldape**[6] | **Caterina Giannini**[1,7]

[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

[2]Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA

[3]Department of Radiology, Mayo Clinic, Rochester, Minnesota, USA

[4]Department of Neurologic Surgery, Mayo Clinic, Rochester, Minnesota, USA

[5]Division of Neuropathology, Department of Pathology and Immunology, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA

[6]Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA

[7]Department of Biomedical and Neuromotor Sciences (DIBINEM), Alma Mater Studiorum, University of Bologna, Bologna, Italy

Correspondence
Caterina Giannini, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA.
Email: giannini.caterina@mayo.edu

Abstract

Adult primary leptomeningeal gliomatosis (PLG) is a rare, rapidly progressive and fatal disease characterized by prominent leptomeningeal infiltration by a glial tumor without an identifiable parenchymal mass. The molecular profile of adult PLG has not been well-characterized. We report the clinical, pathological, and molecular findings of six adult PLG patients (five males and one female), median age 58 years. All cases exhibited pathological leptomeningeal enhancement at presentation. Leptomeningeal biopsy was diagnostic in five (of six) cases, revealing infiltration by an astrocytic glioma with mitotic activity, lacking microvascular proliferation or necrosis. One case was diagnosed at autopsy. All tumors were IDH-wildtype, with five harboring *TERT* promoter mutations. Additional mutations identified were *PTEN* in one case, *TP53* in two cases, and *NF1* in two cases. A chromosome profile with +7/−10 was found in four cases, whereas the remaining two showed either chromosome 7 or 7p gain only. Four cases showed chromosome 9p loss with *CDKN2A*/*B* homozygous deletion, one case showed hemizygous *CDKN2A*/*B* loss, and one case showed intact chromosome 9 and *CDK4*/*GLI1* amplification. DNA methylation profiling was performed in four cases and revealed a match to glioblastoma (GBM) family and mesenchymal typical class with high confidence scores in two cases; the other two cases showed only suggestive combined scores for GBM family and mesenchymal atypical class. The molecular profile of all cases closely aligned with that of adult-type GBM, IDH-wildtype, CNS WHO grade 4. All patients succumbed to the disease. In five cases with extensive leptomeningeal disease at diagnosis, the course was rapid, with median survival of 24 days following palliative care. Only one case, with relatively localized disease at diagnosis, received chemoradiation therapy and survived 535 days, raising the possibility that early diagnosis and timely treatment could improve outcome. A detailed list of previously reported cases is provided in a supplementary table.

KEYWORDS

chromosomal microarray, genome-wide methylation profile, glioblastoma IDH-wildtype, primary leptomeningeal gliomatosis

1 | INTRODUCTION

Leptomeningeal gliomatosis (LG) is characterized by extensive dissemination of neoplastic glial cells in the subarachnoid space and can occur either in isolation without a primary parenchymal lesion (primary LG, PLG) or in association with a parenchymal glioma (secondary LG, SLG). Some glial tumors, primarily ependymomas, are prone to leptomeningeal dissemination; however, leptomeningeal dissemination can occur in almost any glial tumor, particularly late in the course of the disease [1]. Primary presentation of a glioma with widespread involvement of the leptomeninges in the absence of a parenchymal mass is rare. The cell origin is thought to be arising from ectopic leptomeningeal glial cells [2]. In children, primary widespread involvement of the leptomeninges is often seen with diffuse leptomeningeal glioneuronal tumors (DLGNTs), a rare tumor type first recognized as a distinct tumor type in the 2016 WHO classification [3–5].

In adults, PLG is even less common, with less than 50 cases reported since the initial description by Moore in 1954 [6]. In a comprehensive literature review, we found 50 adult PLG cases (age 40 years or older) published largely as case reports or small series. A complete list of references is provided in Table S1. Patients typically present with diffuse leptomeningeal enhancement on imaging, with rapid disease progression frequently leading to death within 6 months. Many of the reported cases, especially the earlier ones, were diagnosed at autopsy. Pathologically, more than half of the cases exhibited an astrocytic phenotype, typically grade 3 or 4 and only infrequently low grade. A few cases were reported as high-grade oligodendroglioma [7, 8] or oligoastrocytoma [9, 10], and very few cases were diagnosed as glioneuronal tumors [4, 11].

Molecular characterization of adult PLG is very limited, with few single molecular tests performed in most cases. In the published adult PLG cases, fluorescence in situ hybridization analysis was conducted on seven cases, revealing one case with a 1p deletion [7], one case with a 19q deletion [12], and two cases with a 1p/19q co-deletion [8, 13]. Additionally, two cases showed H3K27M nuclear positivity in tumor cells [12, 14], two cases had a *TERT* promoter mutation [15, 16], and two cases showed *MGMT* promoter methylation [15, 17]. Here, we report six cases of adult PLG. We documented their clinical, imaging, and pathological features and performed next-generation sequencing (NGS), chromosomal microarray, and whole-genome methylation analysis to better characterize the molecular features of adult PLG.

2 | MATERIALS AND METHODS

2.1 | Patients and pathological review

We identified six patients with a diagnosis of adult PLG from Mayo Clinic ($n = 5$) and Washington University in St. Louis School of Medicine ($n = 1$). The project was approved by the Institutional Review Board. All studies were conducted in accordance with the ethical standards of the Declaration of Helsinki.

The patients included five males and one female, with a median age of 58 years (45–68 years). Clinical history and imaging data were reviewed. All patients underwent leptomeningeal biopsy, including frontal lobes ($n = 2$), temporal lobe ($n = 1$), thoracic spinal cord ($n = 1$), and cauda/filum ($n = 2$). Autopsy was performed in two cases with family consent.

The archived H&E and immunohistochemical stains, performed at the time of the diagnosis were retrospectively reviewed in all cases. The available immunohistochemical stains included GFAP ($n = 5$), OLIG2 ($n = 3$), S100 ($n = 5$), Ki-67 ($n = 3$), H3K27M ($n = 1$), H3K27me3 ($n = 1$), and IDH-R132H ($n = 4$).

2.2 | Next generation sequencing

NGS was conducted using Mayo Clinic neuro-oncology panels ($n = 4$) as previously outlined [18] or by Caris (full exome sequencing including exome-karyotype, $n = 1$). In short, amplicon-based ($n = 1$) or hybrid capture ($n = 3$) NGS libraries were prepared using the Mayo clinic custom neuro-oncology panel consisting of a DNA-based 118-gene ($n = 3$) or 89-gene ($n = 1$) mutation subpanel and an RNA-based 81-gene fusion/transcript variant subpanel. Sequence alterations were categorized using the AMP/ASCO/CAP guidelines [19]. Tiers I/II variants were considered as clinically relevant mutations.

2.3 | Chromosomal copy number variation analysis

Genome-wide chromosome microarray (CMA) analysis was performed for cases 1–5 as previously described [20]. Briefly, DNA was extracted from 5-micron formalin-fixed paraffin-embedded (FFPE) tissue sections using the QIAamp DNA FFPE Tissue Kit (Qiagen). Genome-wide copy number variations (CNVs) and loss of heterozygosity (LOH) were assessed using the OncoScan CNV Plus Array (Thermo-Fisher Scientific) [21]. This array uses a molecular inversion probe method which targets frequent CNVs across 900 cancer-related genes, with a 50–100 Kbp resolution. Raw data were analyzed using the ChAS software (Thermo-Fisher Scientific, Waltham, MA, USA). Whole chromosomes were classified as gained or lost if at least 90% of probe signals from both arms were above or below the threshold. For acrocentric chromosomes (13, 14, 15, 21, 22), only changes in the q-arm were considered.

For case 6, the CNV profile was derived from DNA methylation array (see section below).

2.4 | DNA methylation array profiling

DNA methylation array profiling was conducted at NIH as previously outlined [22]. Genomic DNA was extracted from FFPE tissue and underwent bisulfite conversion using the EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA) and restoration with the Infinium FFPE DNA Restore kit (Illumina, Carlsbad, CA, USA). The DNA was then assayed using the Infinium MethylationEPIC v2 kit (Illumina, Carlsbad, CA, USA) following the Infinium HD FFPE Methylation Assay protocol. Raw idat files were used for classification with the NCI/Bethesda CNS tumor classifier v2. For unsupervised hierarchical clustering, the most significant probes were clustered and visualized using a Uniform Manifold Approximation and Projection (UMAP) plot for dimensionality reduction. *MGMT* promoter methylation status was estimated using the MGMT-STP27 prediction model [23]. Methylation classes were interpreted as "matched" (scores >0.9 for superfamily and class), "no match" (superfamily or class scores <0.5) and "suggestive" (all other score combinations for superfamily and class). UMAP clustering was visually estimated.

3 | RESULTS

3.1 | Clinical findings

Clinical information of all cases is summarized in Table 1. Patients initially presented with a variety of neurological symptoms, including aphasia, extremity pain or weakness, nausea/emesis, nystagmus, and altered mental status. One patient (case 2) was initially diagnosed with suspected Lyme disease due to fluctuating fever, sweats, and diffuse myalgias and was empirically treated with doxycycline.

Imaging was performed in all the cases and showed extensive leptomeningeal enhancement without parenchymal involvement. Figure 1 illustrates the imaging findings of three representative cases. In case 1, at the time of presentation, leptomeningeal enhancement primarily involved the left temporal and frontal lobes (Figure 1A). This evolved after 3–6 months, showing linear and nodular leptomeningeal enhancement coating the nerves of the cauda (Figure 1B,C) and subsequently (10 months later) along the left superior cerebellar folia (Figure 1D). One month before death, significant enhancement was observed in the spinal cord despite treatment (Figure 1E). In case 2, at diagnosis, diffuse leptomeningeal enhancement was present throughout the spinal canal, surrounding the cervical and thoracic spinal cord and cauda equina, extending to the posterior fossa, and involving the cerebellum (Figure 1F–I). Enlargement of the 4th ventricle consistent with communicating hydrocephalus was also noted (Figure 1I). In case 5, irregular leptomeningeal enhancement was present at the dorsal midbrain and tentorium cerebelli (Figure 1J,K) and along the entire cervical and thoracic cord (Figure 1L,M), with extensive leptomeningeal enhancement over the conus (Figure 1N).

Cerebrospinal fluid (CSF) analysis was performed in all patients. Tumor cells were not identified in any case. However, case 4 showed atypical cells suspicious for malignancy. Cases 1, 2, and 5 demonstrated mildly to moderately elevated total protein levels (194–432 mg/dL), whereas cases 4 and 6 had markedly elevated total protein levels (1250 and 1276 mg/dL, respectively). Total

TABLE 1 Clinical information of six adult primary leptomeningeal gliomatosis cases.

Case	Sex	Age (years)	Presentation	Imaging	CSF	Biopsy site	Treatment	Outcome (DOD)
1	M	60	Aphasia, partial seizure	Leptomeningeal enhancement—left temporal & frontal lobes	Negative for tumor cells	Temporal	Radiation & TMZ	535
2	M	56	Back and leg pain	Diffuse leptomeningeal enhancement throughout the spine and brain base	Negative for tumor cells	Cauda/filum	Palliative	10[a]
3	M	66	Altered mental status, aphasia	Diffuse leptomeningeal enhancement—bilateral parafalcine frontal lobes	Negative for tumor cells	Frontal	Palliative	24
4	M	68	Flaccid paraparesis	Plaque-like and nodular enhancement along thoracic cord and cauda equina leptomeninges	Atypical cells suspicious for malignancy	Cauda/filum	Palliative	28
5	F	52	Leg weakness	Diffuse leptomeningeal enhancement throughout the entire spine	Negative for tumor cells	Thoracic	Palliative	76
6	M	45	Back pain	Diffuse leptomeningeal enhancement of spine, cerebral and cerebellar hemispheres, brainstem, and cranial nerves	Negative for tumor cells	Frontal (negative for malignancy)	Immunomodulating therapy, steroids, plasma exchange	22[a]

Abbreviations: CSF, cerebrospinal fluid; DOD, Dead of Disease Survival post-biopsy (days); TMZ, temozolomide.
[a]Autopsy performed.

nucleated cell counts were elevated in cases 4, 5, and 6 (22–154 cells/μL).

After biopsy and pathological diagnosis, Patient 1 underwent radiation and chemotherapy (temozolomide, TMZ) and survived for 535 days (Table 1). The remaining patients who did not receive chemoradiation experienced rapidly fatal outcomes. The median survival time of these five patients post-biopsy was 24 days.

3.2 | Pathological findings

All patients underwent leptomeningeal biopsy ($n = 6$). In five patients, the biopsy showed infiltration by an astrocytic glioma, whereas in one patient (case 6) the frontal leptomeningeal biopsy was non-diagnostic.

The biopsy findings are shown for two representative cases in Figure 2. In case 1, the leptomeninges from the

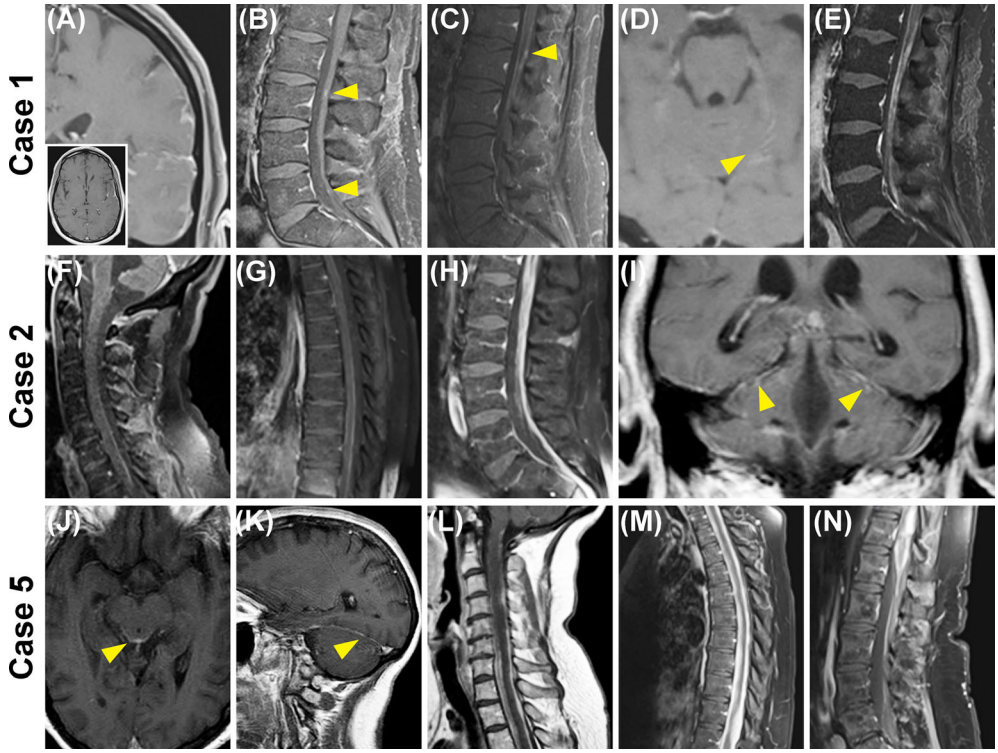

FIGURE 1 Imaging findings of primary leptomeningeal gliomatosis cases. T1-weighted contrast-enhanced MRI of the brain and spinal cord of case 1 (A–E: A, at diagnosis; B, 3 months post-diagnosis; C, 6.5 months post-diagnosis; D, 10 months post-diagnosis; E, 1 month before death), case 2 (F–I) and case 5 (J–N) at diagnosis. In case 1, yellow arrowheads highlight nodular leptomeningeal enhancement along the spinal cord (B, C) and new leptomeningeal enhancement along the left superior cerebellar folia (D). In case 2, yellow arrowheads highlight leptomeningeal enhancement in the cerebellum (I). In case 5, the yellow arrowheads indicate nodular enhancement at the dorsal midbrain (J) and tentorium cerebelli (K).

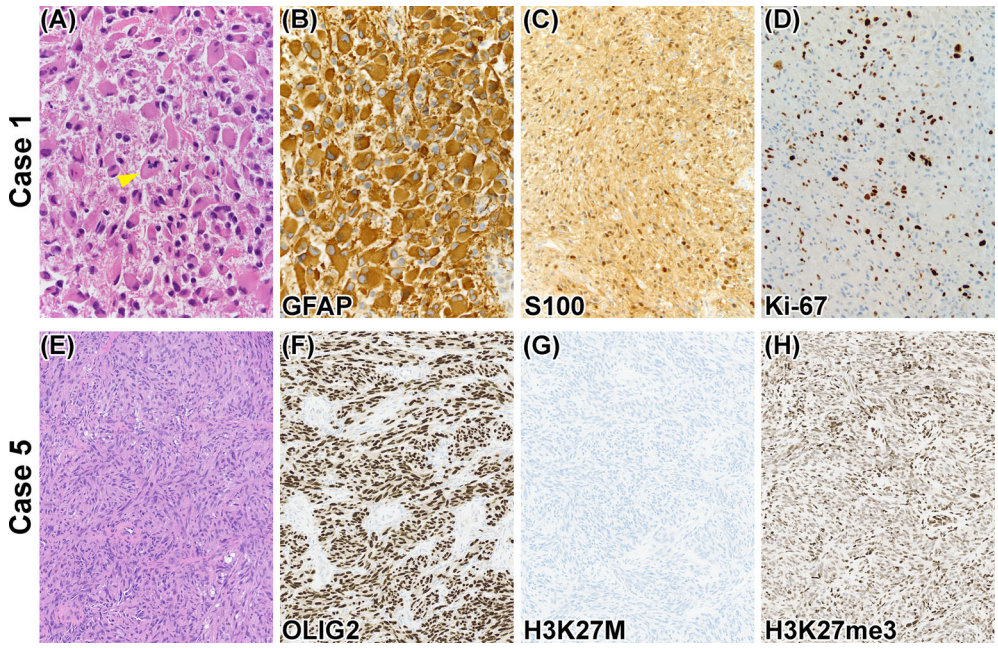

FIGURE 2 Pathological findings of primary leptomeningeal gliomatosis in cases 1 and 5. Leptomeninges from the left temporal lobe biopsy of case 1 are shown with H&E (A) and immunohistochemical (IHC) staining for GFAP (B), S100 (C), and Ki-67 (D). Yellow arrowhead highlights a mitotic figure in (A). Leptomeninges from the thoracic spinal cord biopsy of case 5 are shown with H&E (E) and IHC staining for OLIG2 (F), H3K27M (G), and H3K27me3 (H).

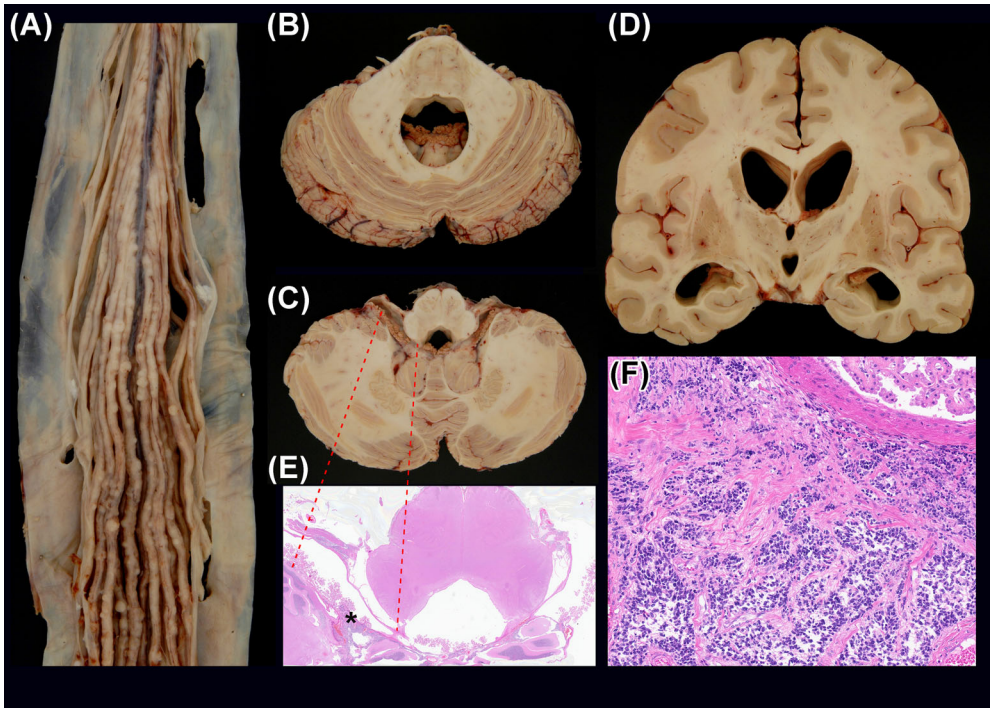

FIGURE 3 Autopsy findings of primary leptomeningeal gliomatosis in case 2. Gross findings of the cauda equina (A), an axial section at the level of the basilar pons (B) and medulla (C), and a coronal section at the level of the mammillary bodies (D). H&E staining of the medulla is shown in (E), with (F) displaying a higher magnification of the area marked by * in (E).

left temporal lobe biopsy showed tumor cells with gemistocytic features and easily identifiable mitoses (Figure 2A), strong and diffuse GFAP staining (Figure 2B), focal S100 staining (Figure 2C), and a brisk Ki-67 index (Figure 2D). In case 5, the leptomeninges from the thoracic spinal cord biopsy showed tumor cells with spindled morphology (Figure 2E), diffusely positive for OLIG2 (Figure 2F), negative for H3K27M (Figure 2G), with retained H3K27me3 expression (Figure 2H), excluding diffuse midline glioma, H3K27-altered. There was neither evidence of microvascular proliferation nor necrosis. The histopathological features of both cases were in the limits of a "grade 3 astrocytoma."

Regarding the other cases, the biopsy of case 2 from the cauda/filum showed diffusely infiltrating tumor cells with oval to elongated nuclei and fibrillar glial processes, morphologically consistent with "grade 3 astrocytoma." The infiltrating tumor cells were positive for GFAP and S100, with a brisk Ki-67 index of 30%–40%. The biopsy of case 3 from the left frontal lobe showed a spindle cell neoplasm diffusely infiltrating the leptomeninges with high mitotic activity (four mitoses per 10 high-power fields) and an elevated Ki-67 index (focally 10%; Figure S1). Necrosis was absent. Tumor cells were positive for GFAP and S100 but negative for OLIG2. The biopsy of case 4 from the cauda/filum meninges showed pleomorphic malignant neoplastic cells multifocally involving the spinal meninges (Figure S1). The tumor cells were negative for GFAP, S100, and OLIG2.

Autopsy of brain and spinal cord were performed in two cases. In case 2, on gross examination, nodular involvement of the tumor was observed in the cauda equina roots of the spinal cord (Figure 3A). Leptomeningeal involvement extended up to the brainstem and involved the foramen of Luschka bilaterally (Figure 3C). There was dilation of the fourth ventricle (Figure 3B) and the lateral ventricles (Figure 3D), consistent with communicating hydrocephalus as observed on imaging (Figure 1I). Histological examination of the medulla showed tumor cells obstructing the foramen of Luschka (Figure 3E,F). In case 6, diffuse intracranial and spinal leptomeningeal involvement, as well as ependymal involvement, were present. The pineal gland and the optic chiasm were encased by thickened leptomeninges (Figure S2A) as was the cauda equina (Figure S2B). Histologically, the tumor filled the subarachnoid with focal extension into Virchow-Robin spaces (Figure S2C,D). There was necrosis in the subependymal regions of tumor, but not in the leptomeningeal component of tumor. Tumor invasion into the cortex was noted in the left occipital lobe. The lumbar spinal cord and nerve roots were encased by tumor, with infiltration into the endoneurium of cauda equina nerve roots (Figure S2E,F).

3.3 | Molecular findings

The molecular profile of the tumors is summarized in Table 2, whereas chromosome microarray and DNA methylation profiling findings are summarized in Figures 4 and 5.

All tumors were IDH-wildtype, and all five (of five) evaluated for histone H3 mutations were also H3-wildtype. Five (of six) cases harbored *TERT* promoter mutation (C228T in four cases and C250T in 1).

TABLE 2 Molecular profiles of six adult primary leptomeningeal gliomatosis cases.

Case	IDH1/2	*TERTp*	Additional mutations	Chr. 7 and 10	*CDKN2A/B*	*MGMTp* methylation	WHO 2021
1	wt	c.-124C>T (C228T)	Not done	+7/−10	Homo del	Not done	GBM, IDH-wt
2	wt	c.-124C>T (C228T)	Not done	+7/−10	Intact (CDK4/Gli ampl)	Negative	GBM, IDH-wt
3	wt	c.-146C>T (C250T)	Not identified	+7	Hemi del	Not done	GBM, IDH-wt
4	wt	wt	TP53 p.C229* TP53 p.R282Afs*55 PTEN p.T319*	+7/−10	Homo del	Negative	GBM, IDH-wt
5	wt	c.-124C>T (C228T)	NF1 p.R461*	+7p	Homo del	Positive	GBM, IDH-wt
6	wt	c.-124C>T (C228T)	NF1 p.W2317*	+7/−10	Homo del	Positive	GBM, IDH-wt

Abbreviations: GBM, glioblastoma; wt, wild type.

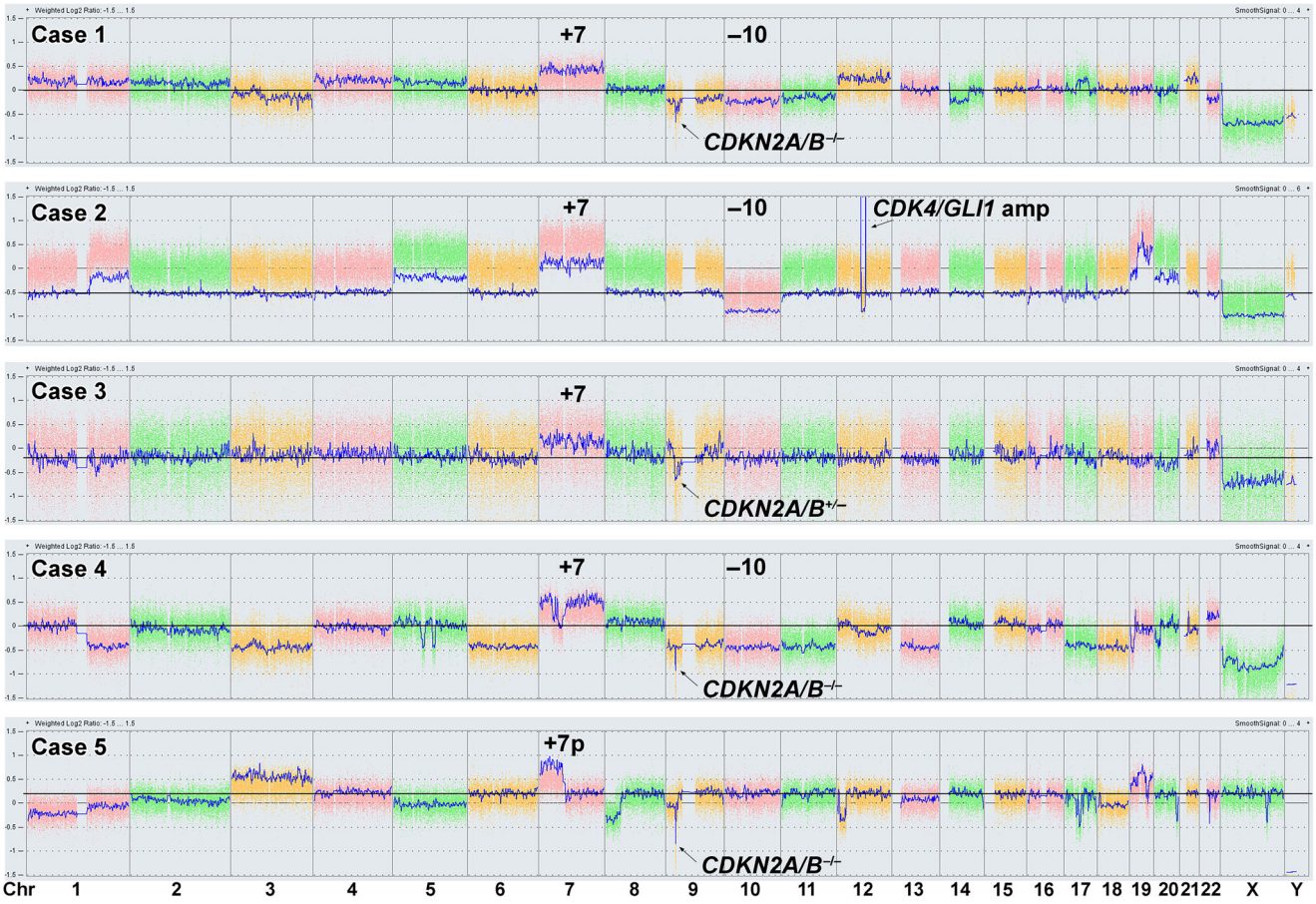

FIGURE 4 Chromosomal microarray findings of primary leptomeningeal gliomatosis cases. Single nucleotide polymorphism (SNP) microarray showing copy number variations using weighted $\log_2$ ratios for case 1 through case 5. Chr, chromosome.

Case 4, lacking *TERT* promoter mutation, harbored two *TP53* mutations (p.C229* and p.R282Afs*55) and one *PTEN* mutation (p.T319*). Two cases (cases 5 and 6) additionally had *NF1* mutations (p.R461* and p.W2317*).

Chromosome 7 gain/10 loss (+7/−10) was present in four cases, whereas two cases showed either chromosome 7 or 7p gain (cases 3 and 5, respectively). Chromosome 9p loss, with *CDKN2A/B* homozygous deletion, was present in four cases, whereas case 3 showed *CDKN2A/B* hemizygous loss. Case 2, with intact chromosome 9, instead showed evidence of *CDK4/GLI1* amplification. Additional full and partial chromosome gains and losses were present (Figure 4).

Taken together, the molecular profile in all cases corresponded to the typical profile of glioblastoma, IDH-wildtype, CNS WHO grade 4 according to WHO 2021 classification of CNS tumors.

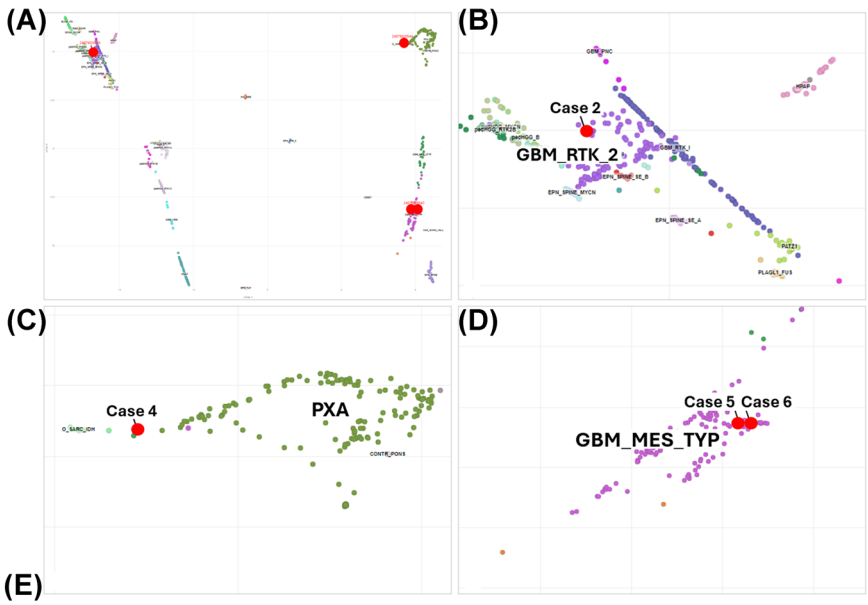

FIGURE 5 EPIC methylation array findings of primary leptomeningeal gliomatosis cases. Uniform Manifold Approximation and Projection analysis of Cases 2, 4, 5, 6 (A–D) and summary of family, family score, class, and class score for each case (E). GBM, glioblastoma.

(E)

Case No.	Family	Family Score	Class	Class Score
Case 2	GBM	0.985 (Match)	GBM_MES_TYP	0.938 (Match)
Case 4	GBM	0.705 (Suggestive)	GBM_MES_ATYP	0.988 (Suggestive)
Case 5	GBM	0.986 (Match)	GBM_MES_ATYP	0.811 (Suggestive)
Case 6	GBM	0.930 (Match)	GBM_MES_TYP	0.930 (Match)

The results of DNA methylation profiling available in 4 cases are summarized in Figure 5, including the results of the NCI/Bethesda CNS tumor classifier v2 and UMAP analysis. Cases 2 and 6 showed a match to the "GBM family, class mesenchymal typical" with a high confidence score (>0.9), whereas the other cases the combined scores were suggestive for the "GBM family, class mesenchymal atypical."

On the UMAP, case 2 was instead "close" to "GBM receptor tyrosine kinase (RTK) II" cluster, whereas case 6 clustered with "Glioblastoma, IDH wild type, mesenchymal typical." Among the cases with suggestive classifier results, case 4 was "near" pleomorphic xanthoastrocytoma cluster and case 5 clustered with class "mesenchymal typical" rather than "mesenchymal atypical."

MGMT promoter methylation status was available in four cases, with two cases (cases 5 and 6) positive for methylation and two cases (cases 2 and 4) negative.

4 | DISCUSSION

Adult PLG is a rare and aggressive glial tumor with a poor prognosis. In the 50 published adult PLG cases, the median survival was only about 6 months post-diagnosis. In our study, consistent with the literature, the median survival post-biopsy was also very short. PLG, particularly in adults, often presents significant diagnostic challenges, as patients often initially develop non-specific neurological symptoms that are difficult to distinguish from more common conditions such as chronic meningitis or inflammatory conditions such as sarcoidosis [12, 13, 24–26]. Imaging of adult PLG often shows diffuse leptomeningeal enhancement (Table S1); however, early stages may present as focal, plaque-like, or nodular enhancement, with spread to other brain or spinal cord regions occurring at later stages, as seen in our case 1. In most reported adult PLG cases where CSF analysis was performed, the majority (34 of 43) were negative for atypical or tumor cells, with some cases ($n = 6$) showing atypical cells, similar to our case 4, and only a few ($n = 3$) highly suggestive or positive for malignancy. Even with leptomeningeal biopsies in the reported cases, a high percentage of initial biopsies were non-diagnostic (nine of 36) as seen in our case 6, necessitating repeated biopsies (Table S1). Primary LG patients have often been treated empirically with anti-tuberculosis regimens and steroids initially [27–30]. Once patients exhibited full-blown neurological symptoms and diffuse leptomeningeal enhancement, and diagnosis was confirmed via biopsy, many were treated palliatively with comfort care, resulting in rapid progression to death. The definitive diagnosis was sometimes made only at autopsy, as in our case 6. This raises the question of whether survival could be improved by prompt diagnosis. Indeed, in most of our cases (five of six), the diagnosis was relatively delayed, and when finally confirmed, the leptomeningeal spread was advanced. Only in one case (case 1), presentation with partial seizure and aphasia led to early discovery of the tumor while still focal. This patient, who underwent

aggressive treatment with radiation and TMZ, is our longest survivor (535 days).

In our study, adult PLG exhibits morphological features of high-grade glioma, including cytologic anaplasia, increased mitoses and brisk Ki-67 index, histologically consistent with at least "grade 3" on biopsies. Among the reported adult PLG cases, while many astrocytomas were histologically consistent with "grade 3," several showed "grade 4" astrocytoma, with tumor exhibiting microvascular proliferation and/or necrosis [31–33]. Most of our cases, similar to what has been reported, showed diffuse expression of glial markers (GFAP and S100). The only case (case 4) lacking expression of GFAP and OLIG2, in the small biopsy examined, showed molecular features of a GBM, IDH-wildtype, including IDH/H3 wild-type status, chromosome 7 gain and 10 loss, and *CDKN2A/B* homozygous deletion (Table 2). Similarly, Yamasaki et al. reported a case with spindle-shaped nuclei that was negative for GFAP, OLIG2, and S100, but harbored a *TERT* promoter mutation c.-124C>T (C228T) and was consistent with a molecular GBM [15].

In our study, multiomics genomic characterization revealed a molecular profile of GBM, IDH-wildtype, CNS WHO grade 4 in all cases. Key features included *TERT* promoter mutations ($n = 5$), a chromosome profile with +7/−10 ($n = 4$), and *CDKN2A/B* homozygous deletion ($n = 4$). DNA methylation profiling in 4 cases further demonstrated an epigenetic profile matching to or being suggestive of that of GBM. Also, the *PTEN* and *NF1* mutations identified in cases 4, 5, and 6 have been recurrently described in GBM [34]. For the reported adult PLG cases, although some ($n = 5$) are morphologically diagnosed as GBM (Table S1), only one case had been molecularly tested and was found to harbor a *TERT* promoter mutation [15]. Although all our cases lack microvascular proliferation and most cases (five of six) lack necrosis, the molecular profiling of adult PLG, in line with GBM, underpins their aggressive nature and rapid progression. These molecular features are distinct from DLGNT, typically diagnosed in children with diffuse leptomeningeal enhancement [4], and characterized by chromosome 1p deletion and MAPK pathway alterations, most commonly the *KIAA1549::BRAF* fusion [3]. Although the behavior of DLGNT is quite variable, the course is generally significantly less rapid with longer survival.

In summary, we report six cases of adult PLG, all characterized by rapid progression and fatal outcomes. Imaging showed diffuse leptomeningeal enhancement. Morphological features observed in biopsies were consistent with high-grade glioma, often in the limits of "grade 3 astrocytoma," while genomic and epigenetic features aligned with those of molecular GBM. Early diagnosis and timely treatments with TMZ and radiation, unfortunately possible only in one patient, may potentially improve survival. Therefore, a leptomeningeal biopsy is highly recommended whenever PLG is suspected. Recently, Sol et al. [16] reported a case of glioblastoma with primary leptomeningeal localization, where nanopore sequencing of cell-free DNA from CSF revealed a *TERT* promoter mutation, CNV profile alterations with *CDKN2A/B* loss, and a high-confidence classification of glioblastoma, midline subtype (GBM-MID) based on DNA methylation analysis. This approach offers a faster and less invasive diagnostic method for diagnosing PLG.

AUTHOR CONTRIBUTIONS

Yi Zhu and Caterina Giannini contributed to the conception and study design; all authors contributed to the acquisition and analysis of data; Yi Zhu, Emilie Russler-Germain, and Caterina Giannini prepared figures and wrote the first draft; all authors reviewed the final submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Yi Zhu https://orcid.org/0000-0002-1778-8880
Cristiane M. Ida https://orcid.org/0000-0003-2768-2913
Emilie Russler-Germain https://orcid.org/0000-0002-2036-6697
Sonika Dahiya https://orcid.org/0000-0002-5585-0964

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SUPPORTING INFORMATION

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

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