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CASE REPORT

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Expanding clinicopathologic knowledge in high-grade glioma with pleomorphic and pseudopapillary features (HPAP): a report of two cases

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Abstract

High-grade glioma with pleomorphic and pseudopapillary features (HPAP) is a recently identified methylation cluster comprised of relatively circumscribed gliomas enriched for variants in *TP53*, *RB1*, *NF1*, *NF2*, *BRAF* and with a more favorable clinical outcome than IDH-wildtype glioblastoma. Here, we present two cases occurring in young adults, one of which occurred in the background of NF2-related schwannomatosis. Both cases demonstrated characteristic histologic features including ependymoma-like areas (Case #1) and an astroblastoma-like phenotype (Case #2), as well as archetypal pseudopapillary structures and pleomorphic tumor cells. High-grade features were present and pathogenic variants in *RB1* and *TP53* were detected. Cytogenetic analysis revealed aneuploidy involving multiple whole chromosomes, including copy neutral LOH in chromosome 13 (Case #1). Both cases were classified as "no match" using the Heidelberg Brain Tumor Classifier (v12.5 and 12.8). Results from a preliminary classification model ("Bethesda Classifier") were consistent with HPAP. Confirmatory dimensionality reduction (t-SNE) showed clustering within (Case #2) or near (Case #1) the HPAP group. Patient #1 is currently receiving maintenance temozolomide following concomitant chemo-radiotherapy, 10 months post-surgery. Patient #2, treated with temozolomide, remains disease-free at 42 months. Our study highlights additional clinical and pathologic insights into this proposed tumor type and may suggest an association with NF2-related schwannomatosis and evolution from low-grade precursors. These observations support the consideration of HPAP as a distinct clinicopathological entity.

Keywords High-grade glioma with pleomorphic and pseudopapillary features, Ependymoma, Astroblastoma MN1-altered, *TP53* mutation, *RB1* mutation, *NF2* mutation, NF2-related schwannomatosis, Low-grade precursor, Malignant transformation

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Introduction

High-grade glioma with pleomorphic and pseudopapillary features (HPAP) is a recently identified methylation cluster of tumors showing a circumscribed glioma phenotype with diverse histological appearances. This heterogeneity is reflected in the broad range of original diagnoses reported in the original series, including anaplastic ependymoma, astroblastoma, anaplastic pleomorphic xanthoastrocytoma, IDH-wildtype glioblastoma, and polymorphous neuroepithelial tumor of the young [5]. HPAP frequently harbor recurrent mutations in *TP53*, *RB1*, *NF1*, *NF2*, *BRAF*, and exhibit aneuploidy, most commonly monosomy of chromosome 13 [3, 5]. Despite the frequent high-grade morphological features, patients' survival appears significantly longer compared to IDH-wildtype glioblastoma [5]. However, whether HPAP constitutes a distinct clinicopathological entity remains an open question.

Cases presentation

The first case concerns a 14-year-old boy who presented with a 2-day history of severe headache and vomiting associated with left facial paralysis. His medical history included delayed psychomotor development and bilateral hearing loss, and at clinical examination showed two *café au lait* spots on his trunk. Brain magnetic resonance imaging (MRI) revealed a large (57 × 58 × 64 mm) solid-cystic enhancing lesion in the right parietal and occipital lobes, surrounded by a significant edema and causing hydrocephalus with a midline shift to the left. The solid component exhibited very low T2 signal with diffusion restriction, suggesting hypercellularity. It appeared inhomogeneous due to serpiginous vessels, hemosiderin deposits, and calcifications. Scalloping of the cranial vault suggested a long-standing process (Fig. 1A, B). The intra- or extra-axial nature of the lesion was unclear, with ependymoma and meningioma considered in the differential diagnosis. Furthermore, multiple enhancing nodules of varying sizes were detected along cranial and spinal nerves, including bilateral vestibular lesions (Suppl. Figure 1), and intramedullary expansive lesions. These findings, along with clinical features, were consistent with NF2-related schwannomatosis (NF2-SCH), later confirmed genetically by the detection of a novel germline heterozygous *NF2* variant (NM_000268.4: c.963_964insA; NP_000259.1: p.Ala323fsTer9) classified as pathogenic according to the ACMG guidelines [6]. The intraventricular mass was resected leaving a minimal residual nodule within the tumor bed and a diagnosis of high-grade circumscribed glioma NEC with features of the emerging group of HPAP was made. Postoperative treatment was slightly delayed due to infectious complications but included temozolomide chemotherapy and craniospinal irradiation (36 Gy) with a tumor bed boost (18 Gy), resulting in a reduction of the residual nodule. The patient is currently receiving maintenance temozolomide, 10 months after surgery.

The second case concerns a 29-year-old woman presenting with a three-month history of progressively worsening headaches. Brain MRI revealed a mass in the right parietal and frontal lobes, adjacent to the temporal horn of the right ventricle. The lesion had a solid component with a large cystic portion, and demonstrated homogeneous contrast-enhancement (Fig. 1C, D). Perfusion MRI indicated high vascularization. Given the radiological suspicion of ependymoma, a complete surgical resection was performed. The final diagnosis was high-grade glioma IDH-wildtype. The patient was treated with temozolomide and remains disease-free 42 months post-surgery.

On microscopic examination, both cases appeared well-circumscribed with clear demarcation from the surrounding parenchyma. Case #1 exhibited distinct morphological heterogeneity: some regions displayed classic ependymal features, characterized by monomorphic cells arranged in perivascular pseudorosettes; other areas showed pseudopapillary structures, frequently separated by zones of necrosis. Additionally, fascicular architecture was observed in certain regions, along with the presence of giant cells exhibiting marked nuclear pleomorphism. Most of the tumor showed high-grade features (i.e. high mitotic index up to 14 per mm^2, necrosis, and microvascular proliferation). However, a low-grade component, featuring perivascular pseudorosettes and eosinophilic granular bodies, was present at the periphery. There was a focal but marked desmoplasia associated to the involvement of the choroid plexus. The tumor expressed patchy GFAP and focal CD34, whereas it was negative for OLIG2 and showed isolated EMA-positive cytoplasmic dots. Case #2 was composed of cells with rounded/ovoid nuclei and eosinophilic cytoplasm arranged around vessels forming astroblastoma-like pseudorosettes which were intermingled with multinucleated giant cells. Limited areas with pseudopapillary features were also present. The tumor showed geographic and pseudopalisading necrosis and scattered mitoses. The lesion was diffusely positive for GFAP, OLIG2, and CD34, and negative for EMA, synaptophysin and BRAF-mutant. Strikingly, p53 immunostaining was strong and diffuse in both cases, except for the p53-negative low-grade component of case #1. In case #1, Ki67 labelling index ranged between 20% and 40% in the major high-grade component whereas was about 1% in the low-grade areas; in case #2, it was about 5%. The histopathological and immunophenotypic characteristics of the cases are illustrated in Fig. 1E-U. DNA methylation analysis yielded a "no match" result (Heidelberg Brain Tumor Classifier v12.5 and 12.8) in both cases. In case #1, based on the morphology and the

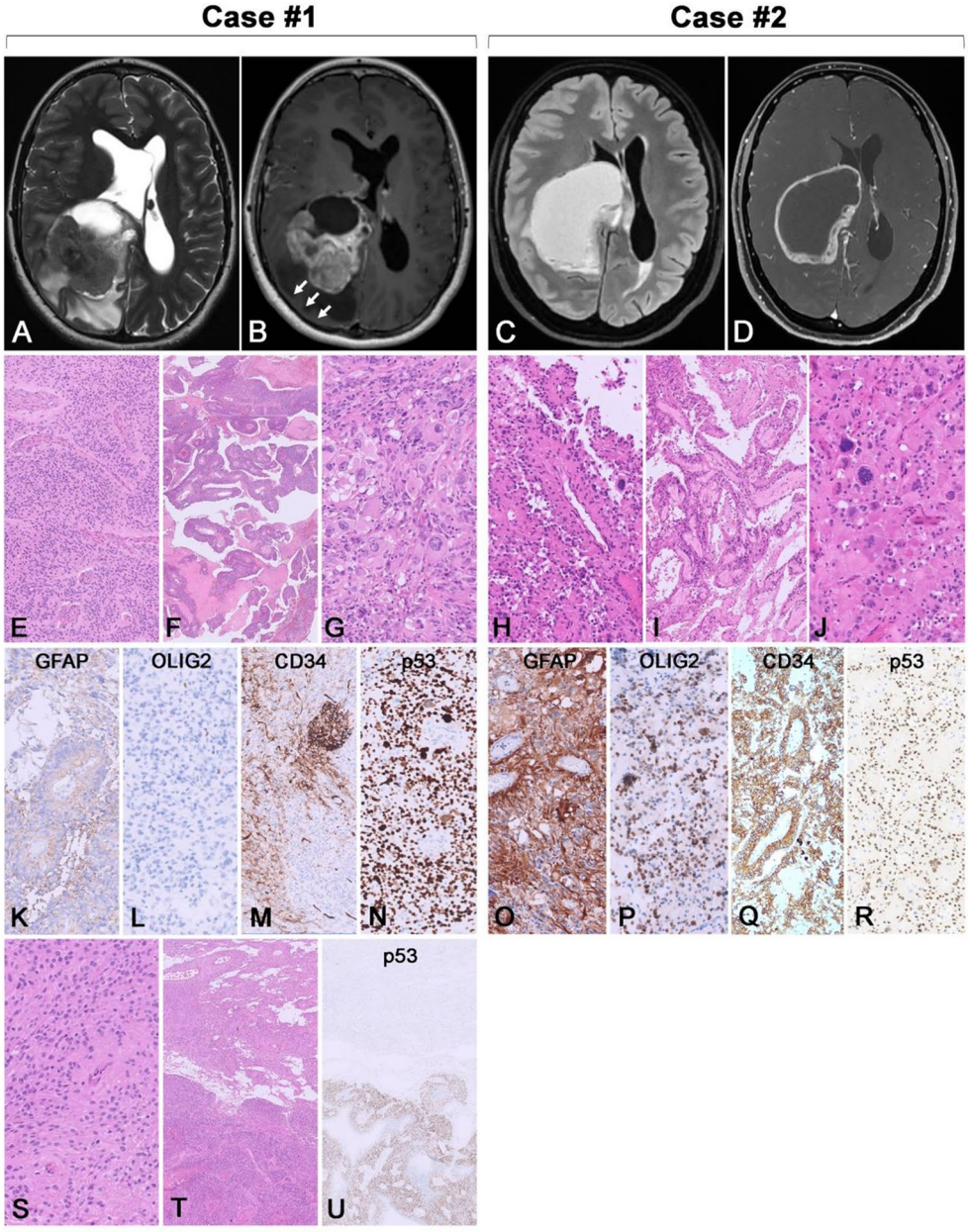

Fig. 1 Radiological and pathological findings in case #1 (A, B, E-G, K-N, S-U) and #2 (C, D, H-J, O-R). Axial T2 TSE (**A**) and post-contrast 3D-T1 SPACE (**B**) images of case #1 show a large, predominantly expansive mass with solid and cystic components in the right parietal and occipital lobes, partially abutting and extending into the right ventricle and causing vasogenic edema, hydrocephalus, and midline shift. The solid component appears hypercellular and demonstrates avid contrast enhancement, as do the cyst walls. Scalloping of the cranial vault is also noted (B, arrows). Axial T2 FLAIR (**C**) and post-contrast T1 (**D**) images show a solid and cystic homogeneously-enhancing lesion in the right parietal and frontal lobes, in close proximity to the temporal horn of the ventricle, associated with midline shift. At histological examination, case #1 displays areas with ependymoma-like features (**E**), areas featuring pseudopapillary structures often separated by necrosis (**F**) and strikingly pleomorphic areas (**G**). Case #2 mostly shows an astroblastoma-like appearance (**H**) with limited pseudopapillary areas (**I**). Intermingled multinucleated giant cells are frequently observed (**J**). Case #1 expresses patchy GFAP (**K**), focal CD34 (**M**) and, in the high-grade component, diffuse p53 (**N**), whereas is negative for OLIG2 (**L**); case #2 is diffusely positive for GFAP (**O**), OLIG2 (**P**), CD34 (**Q**) and p53 (**R**). Only in case #1, mostly at the periphery of the tumor there are areas with a low-grade morphology consisting of monomorphic bland cells featuring perivascular pseudorosettes (**S**); the transition between the low-grade (upper part) and the high-grade (lower part) component is mostly abrupt (**T**); the staining for p53 (**U**) and Ki67 (not shown) highlight the different components of the tumor

NF2-SCH syndromic context, an ependymoma grade 3 with an unusual giant cell/pleomorphic component was first considered. However, data overall (i.e., the pseudopapillary features, the nuclear pleomorphism, the extensive expression of p53 and the result of DNA methylation analysis) suggested the possibility of a HPAP. Case #2 was diagnosed prior to the publication of the original study on HPAP [5], and, although the differential diagnosis included epithelioid glioblastoma, pleomorphic xanthoastrocytoma, and astroblastoma, p53 overexpression combined with the lack of alignment to known methylation classes supported the diagnosis of a high-grade glioma NEC. NGS revealed pathogenic variants in *RB1* and *TP53* in both cases, with variant allele frequencies indicating biallelic inactivation of the genes only in case #1. Additionally, case #1 carried a homozygous variant in *NF2*, and case #2 in *CIC*. Notably, in the low-grade component of case #1 only the *NF2* variant was identified suggesting that the tumor evolved from a *NF2*-driven precursor lesion by inactivating *RB1* and *TP53*. Both tumors exhibited aneuploidy of multiple whole chromosomes, i.e. gains in case #1 and losses in case #2, including loss of chromosome 13 (Fig. 2A, B). Segmental alterations in chromosome 5 and amplification of 1q were also present in case #1. *MGMT* promoter was unmethylated in both cases. SNP analysis conducted only for case #1 showed LOH of 5q21.3q33.3 and multiple whole chromosomes, including 17, 22 and 13 where *TP53*, *NF2*, and *RB1* map respectively (Fig. 2C). No alterations at 9p21 were detected. Only minimal differences in the chromosomal microarray results were identified between the low-grade and the high-grade components of case #1. Molecular findings are summarized in Table 1. t-SNE analysis was performed using selected DFKZ reference classes [1, 2] and the published HPAP cohort [5]: case #2 clustered within the HPAP group, while case #1 localized in close proximity (Fig. 2D). Results from a preliminary classification model ("Bethesda CNS tumor

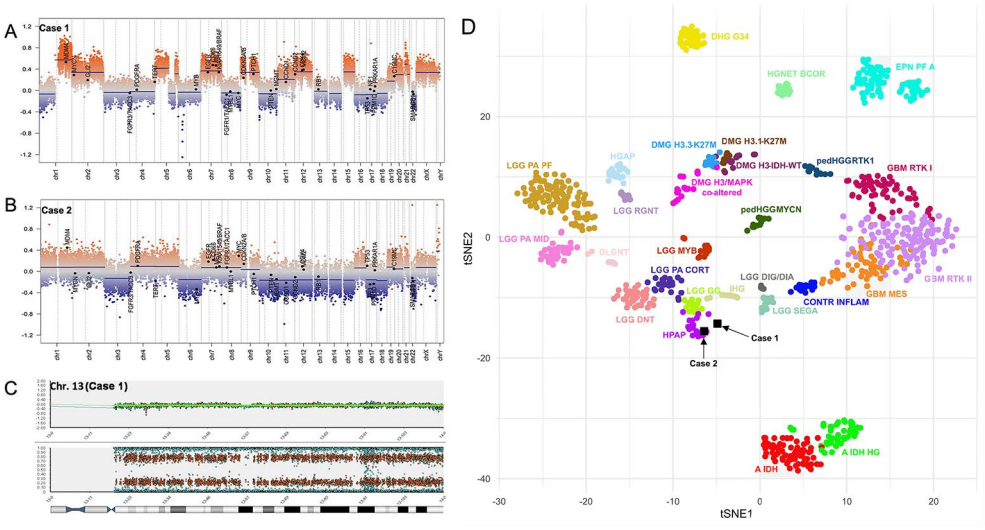

Fig. 2 Copy number alterations (CNA) plot of case #1 (**A**) and #2 (**B**) obtained from DNA methylation analysis. Case #1 shows gain of multiple whole chromosomes (2, 7, 9, 11, 12, 15, 19, 20, 21, X, Y), a 4x gain of 1q and segmental gains at chromosome 5 (**A**). Case #2 shows loss of multiple whole chromosomes (i.e., 3, 6, 10, 11, 12, 13, 14, 15, 17, 18, and 22) (**B**). SNP-analysis shows LOH of multiple whole chromosomes, including chromosome 13 (**C**), where *RB1* maps, and at region 5q21.3q33.3 (not shown). Unsupervised t-SNE analysis of DNA methylation array profile from the current cases (squares) and 1020 previously published brain tumor reference samples [1, 2, 5]: case #2 clusters into the molecular distinct group of HPAP, whereas case #1 clusters close to it (**D**) (A IDH - methylation class IDH glioma, subclass astrocytoma; A IDH HG - methylation class IDH glioma, subclass high grade astrocytoma; CONTR INFLAM - methylation class control tissue, inflammatory tumor microenvironment; DLGNT - methylation class diffuse leptomeningeal glioneuronal tumor; EPN PF A - methylation class ependymoma, posterior fossa group A; DMG G34 - methylation class diffuse hemispheric glioma, H3 G34-mutant; GBM MES - methylation class glioblastoma, IDH wildtype, subclass mesenchymal; pedHGG RTK1- methylation class diffuse paediatric-type high grade glioma, RTK1 subtype; pedHGG MYCN - methylation class diffuse paediatric-type high grade glioma, MYCN subtype; GBM RTK I - methylation class glioblastoma, IDH wildtype, subclass RTK I; GBM RTK II - methylation class glioblastoma, IDH wildtype, subclass RTK II; DMG H3.1-K27M - Diffuse midline glioma, H3.1-K27 mutant; DMG H3.3-K27M - Diffuse midline glioma, H3.3-K27 mutant; DMG H3-WT - Diffuse midline glioma, H3 wildtype; DMG H3/MAPK co-altered - includes three classes: Diffuse midline glioma with EZHIP overexpression and FGFR1 mutant, Diffuse midline glioma H3.3-K27 and BRAF mutant, Diffuse midline glioma H3.3-K27 and FGFR1 mutant; HGAP - High-grade astrocytoma with piloid features; HGNET BCOR - methylation class CNS high grade neuroepithelial tumor with BCOR alteration; HPAP - High-grade glioma with pleomorphic and pseudopapillary features; IHG - methylation class infantile hemispheric glioma; LGG DIG/DIA - methylation class low grade glioma, desmoplastic infantile astrocytoma / ganglioglioma; LGG DNT - methylation class low grade glioma, dysembryoplastic neuroepithelial tumor; LGG GG - methylation class low grade glioma, ganglioglioma; LGG MYB - methylation class low grade glioma, MYB/MYBL1; LGG PA MID - methylation class low grade glioma, subclass midline pilocytic astrocytoma; LGG PA PF - methylation class low grade glioma, subclass posterior fossa pilocytic astrocytoma; LGG PA CORT - methylation class pilocytic astrocytoma; LGG RGNT - methylation class low grade glioma, rosette forming glioneuronal tumor; LGG SEGA - methylation class low grade glioma, subependymal giant cell astrocytoma

Table 1 Molecular abnormalities

Cases	*P*/LP variants* (VAF)	CNA**	LOH regions	RNAseq
#1_High-grade component	*TP53* [NP_000537.3]: p.(Val216Met) (0.75), *RB1* [NP_000312.2]: p.(Cys102Tyrfs*7) (0.79), *NF2* [NP_000259.1]: p.(Ala323Serfs*9) (0.89)	Gain of chr 2, 7, 9, 12, 15, 19, 20, 21, X, Y. Gain at 5p15.33q21.3, 5q33.3q35.3. Gain (4x) of chr 1q	LOH of chr. 1p, 3, 4, 6, 8, 10, 11, 13, 14, 16, 17, 18, 19p, 20, 22, LOH at 5q21.3q33.3	No fusions
#1_Low-grade component	*NF2* [NP_000259.1]: p.(Ala323Serfs*9) (0.89)	Gain of chr 1q, 2, 3, 7, 9, 12, 15, 19, 20, 21, X, Y, Gain at 5p15.33q21.3, 5q33.3q35.3	LOH of chr. 3, 4, 6, 8, 10, 11, 13, 14, 16, 17, 18, 19p, 22, LOH at 5q21.3q33.3	NA
#2	*TP53* [NP_000537.3] p.(Gly262del) (0.22), *RB1* [NP_000312.2] p.(Glu629Argfs*24) (0.38), *CIC* [NP_001291744.1]: p.(Gly1942Val) (0.53)	Loss of chr 3, 6, 10, 11, 12, 13, 14, 15, 17, 18, 22	NA	No fusions

*Gene variants were explored by TruSightOncology500 panel (Illumina) for case #1 and with Oncomine Tumour Mutational Load (TML) (Thermo Fisher) in case #2.
**CNA were investigated with both chromosomal micro-array and DNA methylation analysis in the high-grade component of case #1, with CMA in the low-grade component of case #1 and with DNA methylation analysis in case #2

classifier v2.0") were consistent with an HPAP diagnosis. The superfamily of intermediate_grade_IDH_wild-type_glioma and the methylation class of HPAP were suggested for case #1 (score 0.89 and 0.989 respectively) and matched with case #2 (score 0.984 and 0.991 respectively).

Discussion and conclusions

We herein describe two tumors showing morphological, genetic and epigenetic characteristics overall consistent with the emerging HPAP entity [5], adding to the limited published data. In line with existing literature, both cases displayed features of a circumscribed glioma with perivascular pseudorosettes, a remarkable giant cell/pleomorphic component, pseudopapillary structures and diffuse p53 expression. While both tumors were classified as high-grade, neither patient has shown signs of disease progression to date.

Our findings underscore several noteworthy features of HPAP. In the landmark study, only 1 out of 25 cases with a known anatomical location was intraventricular [5]. Notably, both our cases were closely associated with the lateral ventricles, suggesting that HPAP should be considered in the differential diagnosis of supratentorial lesions located juxta- or intraventricularly.

Loss of chromosome 13 is currently considered the most frequent copy number alteration in HPAP. Interestingly, although case #1 maintained both chromosomes 13, showed copy-neutral LOH of multiple whole chromosomes including chromosome 13, accounting for the biallelic inactivation of *RB1*.

Perhaps the most intriguing finding was the association with NF2-SCH. In the original series, *NF2* mutations were identified in 3 out of 21 tumors tested, although germline data were not available, limiting insights into the underlying genetic context [5]. More recently, a study presented in abstract form reported the occurrence of HPAP in the setting of cancer predisposition syndromes, identifying germline mutations in DNA repair genes in 2 out of 10 patients [4]. Notably, the association with NF2-SCH in our case # may explain several of its distinctive clinicopathological features, including the unusually early age of onset —significantly younger than the median age of 46.5 years reported in the original series [5] —and the apparent progression from a low-grade precursor. Although a small subset of tumors in the original cohort presented as low-grade entities (e.g., polymorphous neuroepithelial tumor of the young), the coexistence of well-defined low-grade and high-grade components, as observed in our case, represents a novel and previously unreported finding. While both components carried *NF2* inactivation, alterations in *TP53* and *RB1* were restricted to the high-grade lesion, likely driving the malignant transformation of the tumor. The morphological features, such as perivascular pseudorosettes, along with the immunophenotypic profile (e.g., GFAP positivity and OLIG2 negativity), suggest that the precursor lesion may be related to a rare *NF2*-inactivated low-grade ependymoma, particularly given its unusual supratentorial intraventricular location.

Data on HPAP are still limited and nowadays the diagnosis of HPAP remains descriptive, i.e. circumscribed high-grade glioma NEC. We decided to treat patient #1 with both radiation and temozolomide considering the high-grade features, the high Ki67 proliferation index of the tumor and *TP53* bi-allelic inactivation. Similarly, patient #2 was treated with temozolomide due to the high-grade aspects of the tumors. Recognizing HPAP as an entity with intermediate behavior despite the high-grade morphology will help the whole community of pediatric and young adulthood oncologists to modify the treatment approach.

Our study brings to light novel aspects of this emerging group of tumors, such as the possible association with NF2-related schwannomatosis syndrome and the evolution from a low-grade precursor, and may contribute to consider HPAP as a clinicopathological entity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40478-025-02017-9.

Supplementary Material 1

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Author contributions
Study conception: S.R., V.B., E.M.; Acquisition of data: S.R., I.G., S.P., A.M., E.P., G.K.R., C.T, G.M., F.A., E.A., A.C., A.M., G.S.C., V.A., E.M., V.B.; Analysis and interpretation of data: S.R., I.G., A.M., S.P., E.M., V.A., E.P., G.S.C., A.M., G.K.R., R.A., C.G., V.B.; Drafting of manuscript: S.R., V.B., E.M.; Critical revision: F.L., R.A., C.G, A.M., I.G., V.A. All authors approved the final version of the manuscript to be published.

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Data availability
NGS data, methylation profiling, chromosomal microarray analysis data are available on request from the authors.

Declarations

Ethics approval and consent to participate
This study was approved by the ethics committee; written consent to the publication of the cases was given by the patient or patient's parents.

Competing interests
The authors declare no competing interests.

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