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Review

Newly recognised Tumour Types in Glioneuronal tumours according to the 5th edition of the CNS WHO Classification

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Summary
Glioneuronal tumours (GNT) are uncommon neoplasms, characterised by glial and neuronal differentiation.
In the 5th edition of the World Health Organization (WHO) Classification, they are grouped under the heading "Glioneuronal and neuronal tumours", which comprises fourteen different tumours, among which the diffuse glioneuronal tumour with oligodendroglioma-like cells and nuclear clusters (DGONC), myxoyd glioneuronal tumour (MGT) and multinodular and vacuolating neuronal tumour (MNVNT) are new types.
MGT and MNVNT are classified WHO grade 1 and may be recognised and diagnosed by peculiar clinical-pathological features. DGONC was not assigned a WHO grade and was only provisionally included among GNT, due to the possibility that it rather represents an embryonal tumour type or subtype. Although the histopathological characteristics may be useful for its identification, the specific methylation profile is an essential diagnostic criterion for DGONC.

Key words: glioneuronal tumours, diffuse glioneuronal tumour with oligodendroglioma-like cells and nuclear clusters, myxoyd glioneuronal tumour, multinodular and vacuolating neuronal tumour

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Introduction

Glioneuronal tumours (GNT) are uncommon neoplasms, accounting for approximately 1-3% of all brain tumours [1] and owing their name to the co-occurrence of glial and neuronal differentiation [2].
In the 5th edition of the World Health Organization (WHO) Classification, GNTs are grouped with neuronal tumours under the heading "Glioneuronal and neuronal tumours", that includes 14 different tumour types (Tab. I) [3].
The recent evidence that some tumours display specific molecular alterations and that different tumour types exhibit a peculiar DNA methylation profile, reflecting their cell of origin and the somatic mutations acquired during tumourigenesis and progression, has strongly contributed to this latest classification of GNTs [4]. Indeed, DNA methylation profiling has clarified that, in spite of similar clinical features, such as the slow growth, association with seizures and favorable outcome[1], and of frequently overlapping morphology, GNTs are epigenetically and biologically different [3]. In addition, epigenetic and genetic studies have allowed the identification, among GNT, of three new tumour types, characterised by a peculiar DNA methylation profile or molecular alterations [3]. These

Table I. Different types of Glioneuronal and neuronal tumours, according to the 5th WHO Classification. Newly Recognized Tumour Types are highlighted in bold.

Glioneuronal and Neuronal Tumours
Ganglioglioma
Gangliocytoma
Desmoplastic infantile ganglioglioma/Desmoplastic infantile astrocytoma
Dysembryoplastic neuroepithelial tumour
Diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters
Papillary glioneuronal tumour
Rosette-forming glioneuronal tumour
Myxoid glioneuronal tumour
Diffuse leptomeningeal glioneuronal tumour
Multinodular and vacuolating neuronal tumour
Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)
Central neurocytoma
Extraventricular neurocytoma
Cerebellar liponeurocytoma

are the diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters, the myxoid glioneuronal tumour and the multinodular and vacuolating neuronal tumour.

This review will focus on these three new tumour types, describing their main clinical, histopathological, immunohistochemical and molecular features, in order to provide possible clues for recognition in routine practice.

Diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters (DGONC)

DGONC was originally described in 2020 by Deng et al. [5], who identified 31 tumours with peculiar morphology and distinct DNA methylation pattern among over 25,000 central nervous system (CNS) neoplasias profiled for genome-wide DNA methylation. Therefore, although its actual incidence is unknown, this tumour is presumed to be exceptionally rare.

DGONC mainly occurs in a paediatric age (median age: 9 years: range: 2-75 years), with no sex predilection, and it localises in the cerebral hemispheres, mainly in the cortical/subcortical area of the temporal lobe [5,6]. Nonetheless, ventricular location was reported in one case [5]. Perhaps due to the lack of a preferential anatomical location, no specific symptoms have been reported.

The name "DGONC" is derived from the peculiar histopathological features, which represent the hallmark of this tumour type: i) a diffuse growth; ii) clear cells with a peri-nuclear halo, mimicking oligodendroglioma; iii) nuclear clusters, i.e. multinucleated cells with nuclei disposed as pennies in a plate [5] (Fig. 1). Additional histopathological features are vascular proliferation and neuropil-like islands.

Suggesting its glioneuronal nature, by immunohistochemistry DGONC is characterised by diffuse positivity for OLIG-2 and synaptophysin [5], focal positivity for Neu-N and MAP2, and negativity for GFAP (Fig. 1; Tab. II) [3,5,6].

The molecular hallmark of DGONC is monosomy of chromosome 14, which is invariably found in all the reported cases [5,6]. No other recurring molecular alterations have been described [5,6].

Due to the low number of cases with an available follow-up, DGONC was not assigned a CNS WHO tumour grade [3]. Mitotic index is variable and Ki-67 labeling index can be up to 30% [3]. Based on the data of 26 patients, the 5-year survival rate is 89% and the progression-free survival is 81% [7]. However, the application of radio- or chemotherapy might have impacted the outcome, modifying the natural history of the disease [6].

According to the WHO Classification [3], the essential diagnostic criteria for DGONC are the demonstration of the specific methylation profile, the histological evidence of oligodendrocyte-like cells and the immunohistochemical positivity for OLIG2 and synaptophysin and negativity for GFAP. However, a WHO caveat is that, if DNA methylation profiling is unavailable, morphological features may provide an approximation.

Myxoid glioneuronal tumour (MGT)

MGTs are uncommon primary brain tumors, usually occurring in children and young adults, with a peak incidence in the second and third decades of life and an equal sex distribution [7-8]. They feature characteristic location in the septal nuclei, septum pellucidum, corpus callosum or periventricular white matter [8].

MGT represents a new tumour type encompassing a large part of previously diagnosed cases of dysembryoplastic neuroepithelial tumors (DNT) and rosette-forming glioneuronal tumors (RFGT) located in septum pellucidum and deep periventricular white matter [9-10].

The most common symptoms include headache, emesis, seizures and behavioral disturbance [7].

At imaging, MGTs are well circumscribed and display T1-hypointensity and T2-hyperintensity, without contrast enhancement or restricted diffusion (Fig. 2). Tumours arising in the septal nuclei and septum pellucidum are frequently associated with obstructive hy-

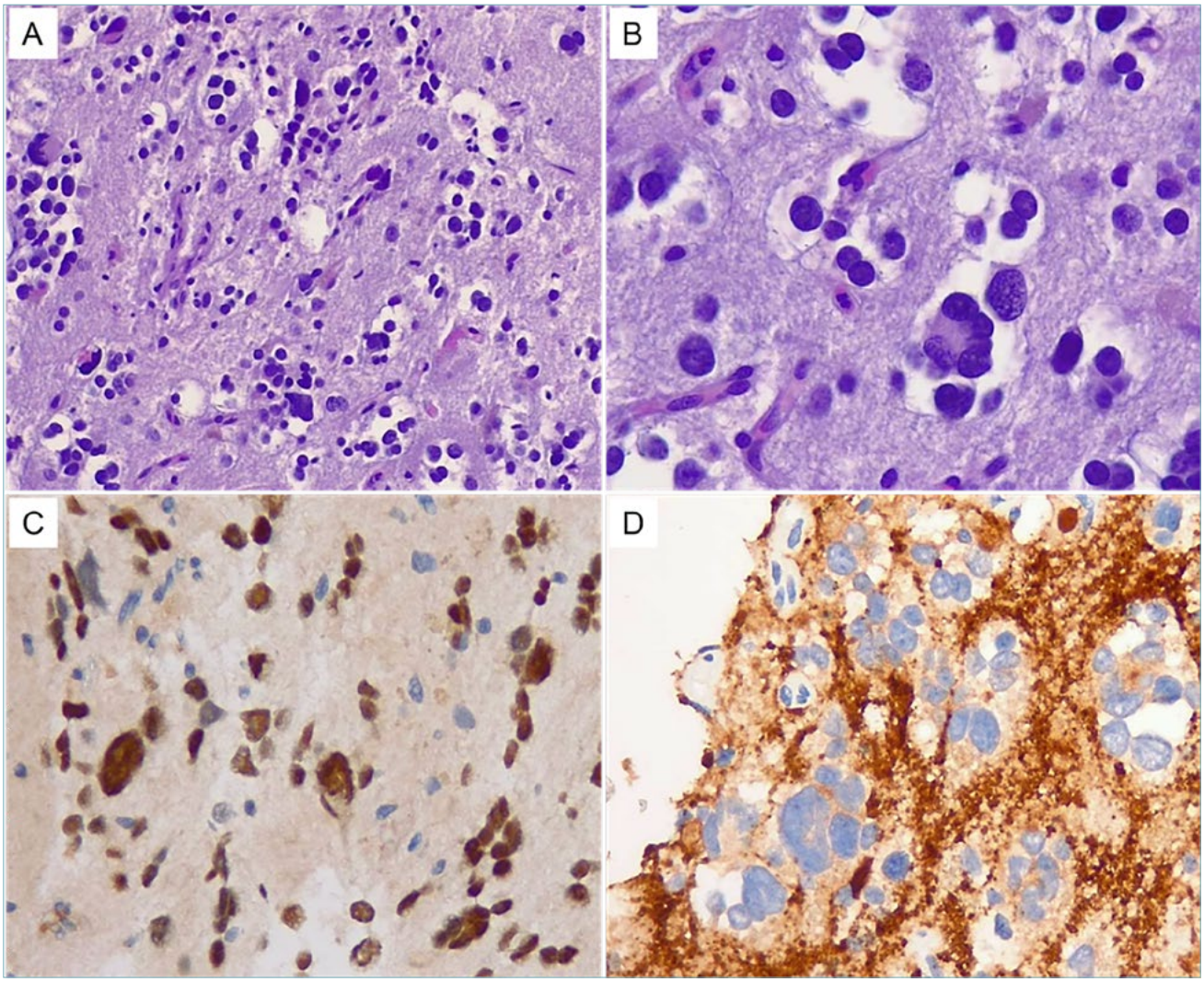

Figure 1. Diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters. (A) The tumour is mildly cellular, composed of medium-size cells with mild pleomorphic oval nuclei along with cells with round nuclei and clear perinuclear halo (H&E, 200X magnification). (B) Multinucleated cells and nuclear clusters composed of large pleomorphic nuclei (H&E, 400X magnification). (C) Olig2 immunostain shows a diffuse nuclear positivity (100X magnification). (D) Neoplastic cells are reactive for Synaptophysin antibody (100X magnification)

drocephalus. Some patients may have disseminated disease throughout the ventricular system at the initial presentation [7-8].

Histologically, MGT is a circumscribed tumour composed of oligodendrocyte-like cells ensheathed in a prominent myxoid stroma (Fig. 2). Some cases may also feature floating neurons and perivascular neuropil, thus resembling DNTs, while other cases may show neurocytic rosettes and perivascular neuropil, similarly to RFGT. Rosenthal fibers and eosinophilic granular bodies are not usually observed, mitoses are very rare or absent [7-8].

The oligodendrocyte-like neoplastic cells of MGTs are immunopositive for OLIG2, SOX10, GFAP and MAP2 and negative for synaptophysin. Floating neurons, perivascular neuropil and neurocytic rosettes are Synaptophysin positive (Fig. 2; Tab. II). CD34 staining is limited to vessels and proliferation index is usually low [7-8].

MGTs are characterised by a recurrent dinucleotide mutation in the *PDGFRA* gene, resulting in an amino acid substitution, either p.K385L or p.K385I. These somatic mutations occur typically in absence of accompanying *PDGFRA* gene amplification [9-11]. At DNA

methylation profiling, MGTs have an epigenetic signature close to that of DNTs of the cerebral cortex [7,8,11].
MGT is classified CNS WHO grade 1 due to its favorable outcome [9,10]. High-grade transformation has not been reported to date. Prognosis remains good even in cases with local recurrence or dissemination throughout the ventricular system [7,8].
According to the WHO Classification [3], the essential diagnostic criteria for MGT are low-grade glioneuronal neoplasm with DNT-like or RGNT-like histologic features and location in septal nuclei, septum pellucidum, corpus callosum or periventricular white matter. Demonstration of *PDGFRA* p.K385L/I dinucleotide mutation is desirable, but not essential.

Multinodular and vacuolating neuronal tumour (MVNT)

MVNT is a benign tumour associated with seizures. Mean age of MVNT patients at diagnosis is 42 years (age range: 5-71), with few pediatric examples and a slight male prevalence (1.5:1 ratio). More than 75% of cases originate in the temporal lobes, followed by the frontal lobes (10-15%) [12-15]. The large majority of patients presents with seizures, mainly of complex partial type with or without secondary generalisation. Other common symptoms are headache, episodic confusion and dizziness [16,17]. To date, factors predisposing to the development of MVNT have not been identified.

Table II. Clinical-pathological and molecular features of the three newly recognised GNTs

Tumour type	Histopathological clues	IHC	Clinical features	Molecular features	Differential diagnosis
DGONC (no WHO CNS grade)	- Oligodendrocyte-like cells - Nuclear cluster - Diffuse growth	- GFAP- - Synaptophysin+ - OLIG 2+	- Mainly paediatric - No sex predilection - No site predilection - No specific symptoms - Prognosis indefinite	- Monosomy 14	- Oligodendroglioma - GBM - Extraventricular neurocytoma
MGT (WHO CNS grade 1)	- Oligodendrocyte-like cells - Prominent myxoid stroma - Floating neurons - Perivascular neuropil - Neurocytic rosettes	- OLIG 2+ - SOX10+ - GFAP+ - MAP2+ - Synaptophysin+	- Mainly children and young adults - No sex predilection - Septal nuclei, septum pellucidum, corpus callosum or periventricular white matter - Seizures - Favorable outcome, but some cases may recur locally or disseminate throughout the ventricular system	- Dinucleotide mutation in *PDGFRA* gene *p.K385L/I*	- DNT - Rosette-forming glioneuronal tumour
MVNT (WHO CNS grade 1)	- Multinodularity - Neuronal constituents - Low-grade features - Tumour cell/ matrix vacuolation	- OLIG 2+ - Doublecortin+ - non-phosphorylated NFP+ - Synaptophysin+ - MAP2+	- Mean age 42 years - Few paediatric cases - Slight male prevalence - Seizures - >75% of cases in temporal lobes - Benign behaviour	- Hotspot mutations and small indels in *MAP2K1* - *BRAF* mutations (excluding *p.V600E* mutations) - *FGFR2* fusions	- Gangliocytoma - Neuronal heterotopia

Abbreviations: DGONC: diffuse glioneuronal tumour with oligodendroglioma-like cells and nuclear clusters. GBM: glioblastoma. MGT: myxoid glioneuronal tumour. DNT: dysembryoplastic neuroepithelial tumour. MVNT: multinodular and vacuolating neuronal tumour.

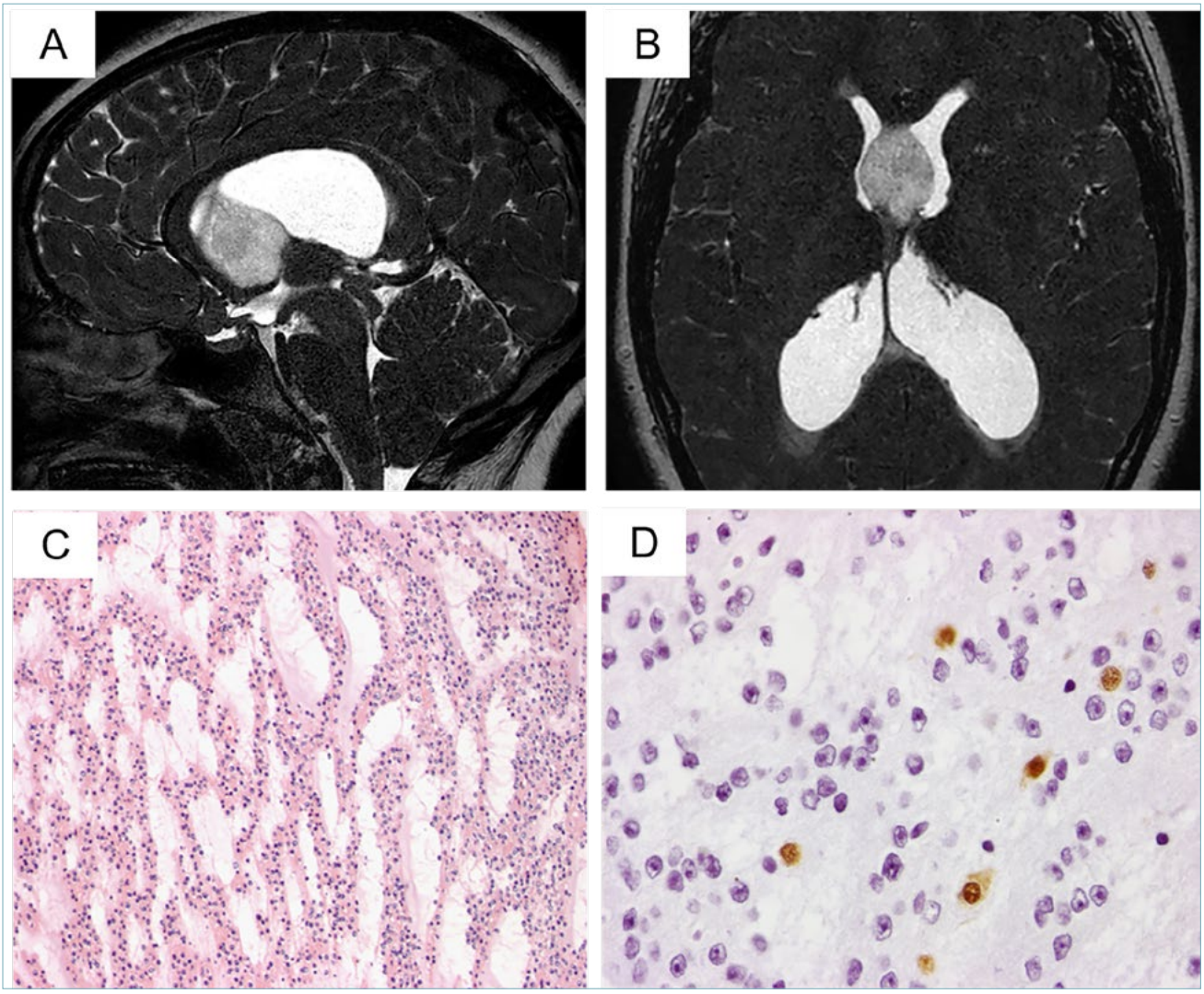

Figure 2. Myxoid glioneuronal tumour. (A) Sagittal and (B) Axial T2-weight MRI. Hyperintense lesion in the anterior portion of the septum pelluciduum resulting in a biventricular hydrocephalus. (C) Histological evaluation shows a tumour characterized by proliferation of oligodendrocyte-like cells embedded in a prominent myxoid stroma, with a delicate capillary network (H&E, 100X magnification). (D) NeuN antibody highlights occasional floating neurons (400X magnification).

On neuroimaging, MVNTs display peculiar features consisting in T2-FLAIR hyperintensity, clustered nodules in the deep cortex and superficial subcortical white matter. Typically associated mass effects, such as oedema or contrast enhancement, are absent [12-15] (Fig. 3).

Histologically, MVNTs feature clear, hypomyelinated nodules showing a fibrillary matrix, prominent vacuolar changes and monomorphic neuronal cells, with round nuclei, evident nucleoli and eosinophilic cytoplasm [12,13,17], whose size ranges from intermediate to large, without reaching the proportions of ganglion elements (Fig. 3). Neoplastic cells are haphazardly distributed or may align along capillary vessels. Rosenthal fibers, eosinophilic granular cells and microcalcifications are usually absent, as are mitotic figures, microvascular proliferation and necrosis [13]. MVNT may be associated with cortical disorganisation [12].

At immunohistochemistry, neoplastic cells are positive for OLIG2, doublecortin and non-phosphorylated NFP and may express synaptophysin and MAP2 [12,13,18] (Fig. 3; Tab. II). They are negative for chromogranin and phosphorylated NFP, with absent or only faint positivity for NeuN. Nodular matrix results intensely positive for α-internexin and weakly for synaptophysin and MAP2 [13,18]. CD34 expression may be observed in

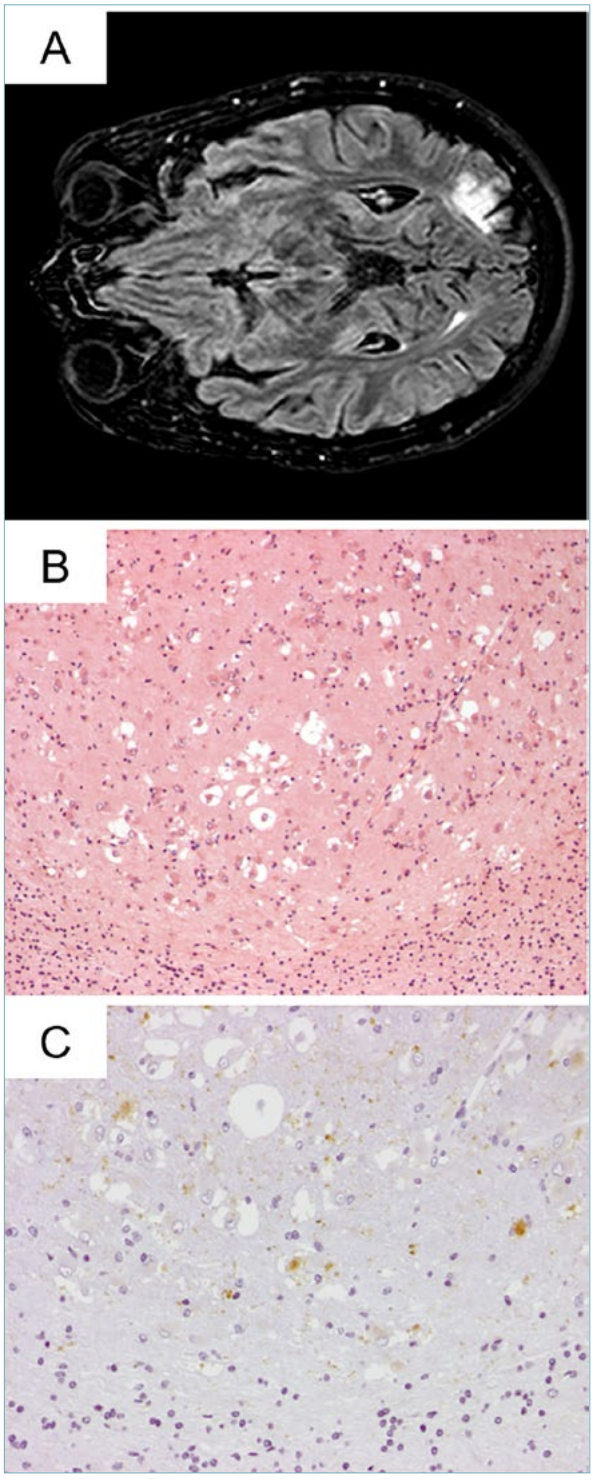

Figure 3. Multinodular and vacuolating neuronal tumour. (A) MRI demonstrates FLAIR-hyperintense clustered nodular lesion, with involvement of cortex and superficial white matter, and absence of mass effect. (B) High-power magnification highlights prominent vacuolar changes and mature-appearing neuronal cells of intermediate to large size (H&E, 200X magnification). (C) Synaptophysin is only weakly and focally expressed (200X magnification).

ramified neural elements and GFAP-positive reactive astrocytes of the associated cortex.

Molecular analyses may reveal MAPK pathway-activating abnormalities, commonly consisting in hotspot mutations and small indels in MAP2K1. *BRAF* mutations or *FGFR2* fusions are less common [3].

MVNT is classified CNS WHO grade 1. It does not recur after gross total resection and tumour residue remains stable in case of subtotal resection [12,13,15].

According to the WHO Classification[3], the essential diagnostic criteria for MVNT are multinodularity, neuronal constituents, low-grade features and tumour cell/matrix vacuolation. Immunophenotypic and molecular issues are desirable, but not essential.

Discussion

GNTs encompass several tumour types that share clinical-pathological features such as the presentation in young patients, preferential localisation in the temporal lobe, association with seizures, indolent behaviour, and histological presence of oligodendrocyte-like cells. However, this group of tumours is heterogeneous, both morphologically and genetically. Although the 2016 WHO classification of CNS tumours introduced an integrated molecular-histopathologic diagnostic approach, this substantially did not involve GNTs [19].

The current 5th edition of WHO Classification published in 2021[3] partly amended this gap, adopting several molecular diagnostic tools that have improved diagnostic reliability and lead to the recognition of three new tumour types in this group. More and more lesions underlying drug-resistant focal epilepsy have extensively been studied by integrated molecular-histological approach and classified [20], in spite of their morphological similarity [21].

Among the newly identified tumour types, only MGT displays a distinctive genetic alteration (PDGFRA p.K385L/I dinucleotide mutation) [9-11], while MVNT features dysregulation of the MAPK pathway due to *BRAF* or *FGFR* alterations [14], similarly to other GNTs, and DGONC does not harbour any recurrent and characterizing genetic abnormality [3,5,6]. Of note, for MVNT and MGT, the WHO Classification considers only clinical-pathological features as essential diagnostic criteria [3], while the demonstration of their molecular alterations is desirable but not required. The reliable classification of these two tumours as low-grade is essential to avoid over-treatment, toxicity and long-term side-effects, particularly in pediatric patients [22], considering that even cases with multifocal CSF dissemination at presentation or at follow-up have a good prognosis [23].

On the other hand, the diagnosis of DGONC requires, not only the appropriate histological and immunohistochemical features, but also a matching DNA methylation profile [3]. This is probably because DGONC displays histopathological features suggestive of several other tumour types, including gliomas or embryonal tumours [5]. Reflecting the challenging histopathological recognition of DGONC, the original diagnoses in the first reported series by Deng et al. [5] were widely variable, and included anaplastic oligodendroglioma, atypical extra-ventricular neurocytoma, dysembryoplastic neuroepithelial tumour, primitive neuroectodermal tumour or glioblastoma, i.e. tumour types with a CNS WHO grade ranging between 1 and 4, and with markedly divergent prognosis. Differently from the two other new tumour types, DGONC has been only provisionally included among GNT [3]. Indeed, while MGT displays a methylation profile close to DNT, reflecting their biological similarity, DGONC forms a distinct methylation cluster close to FOXR2-activated CNS neuroblastoma [3], which suggests it rather represents a type or a subtype of CNS embryonal tumours [3]. Therefore, the recognition of DGONC by its methylation pattern is crucial for a future definitive characterisation.

In conclusion, the epigenetic and genetic profiling of CNS tumours has determined an advance in the classification of GNTs, consisting in the definitive characterization of MNVT as neoplastic, in the separation of MGT by DNT due to a specific genetic alteration and different methylation profile, and to the recognition of DGONC. Although the diagnosis of this latter requires DNA methylation profiling, specific histopathological and immunohistochemical features may be helpful for its recognition.

Conflict of interest

The authors declare no conflict of interest.

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Ethical consideration

Not applicable.

Authors' contributions

Conceptualization: VB and GM; methodology: VB, FG, GM; data curation: VB, FG, GM; writing-original draft preparation: VB, FG, GM; writing-review and editing: VB, FG, GM. All authors have read and agreed to the published version of the manuscript.

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