Pilocytic astrocytoma: The paradigmatic entity in low-grade gliomas (Review)

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Abstract. Among low-grade gliomas, representing 10-20% of all primary brain tumours, the paradigmatic entity is constituted by pilocytic astrocytoma (PA), considered a grade 1 tumour by the World Health Organization. Generally, this tumour requires surgical treatment with an infrequent progression towards malignant gliomas. The present review focuses on clinicopathological characteristics, and reports imaging, neurosurgical and molecular features using a multidisciplinary approach. Macroscopically, PA is a slow-growing soft grey tissue, characteristically presenting in association with a cyst and forming a small mural nodule, typically located in the cerebellum, but sometimes occurring in the spinal cord, basal ganglia or cerebral hemisphere. Microscopically, it may appear as densely fibrillated areas composed of elongated pilocytic cells with bipolar 'hairlike' processes or densely fibrillated areas composed of elongated pilocytic cells with Rosenthal fibres alternating with loosely fibrillated areas with a varied degree of myxoid component. A wide range of molecular alterations have been encountered in PA, mostly affecting the MAPK signalling pathway. In detail, the most frequent alteration is a rearrangement of the BRAF gene, although other alterations include neurofibromatosis type-1 mutations, BRAFV600E mutations, KRAS mutations, fibroblast growth factor receptor-1 mutations of fusions, neurotrophic receptor tyrosine kinase family receptor tyrosine kinase fusions and RAF1 gene fusions. The gold standard of PA treatment is surgical excision with complete margin resection, achieving minimal neurological damage. Conventional radiotherapy is not required; the more appropriate treatment appears to be serial follow-up. Chemotherapy should only be applied in younger children to avoid the risk of long-term growth and developmental issues associated with radiation. Finally, if PA recurs, a new surgical approach should be performed. At present, novel therapy involving agents targeting MAPK signalling pathway dysregulation is in development, defining BRAF and MEK inhibitors as target therapeutical agents.

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Contents

1. Introduction

Low-grade gliomas (LGGs) approximately represent 10-20% of all primary brain tumours; they are more commonly diagnosed in younger individuals with a sporadic occurrence, although their precise aetiology is still not well understood (1). The clinical presentation of LGGs can vary depending on the location and size of the tumour, but commonly, symptoms such as headaches, cognitive impairment, focal neurological deficits and changes in behaviour or personality have been reported (1). It is well known that LGGs can arise in various locations within the brain, including the cerebral hemispheres, brainstem and cerebellum (1). Regarding the prognosis of LGGs, it can vary depending on various factors, such as tumour location, extent of resection, age of the patient as well as molecular characteristics. Nevertheless, their behaviour is generally more favourable, mainly compared to high-grade gliomas, with median survival ranging from 4.7 to 9.8 years (1).

The paradigmatic LGG subtype is represented by the pilocytic astrocytoma (PA), which is considered a grade 1

tumour by the World Health Organization (WHO). Herein, we will focus on the clinicopathological, neurosurgical and molecular features of this rare entity through a multidisciplinary approach, also taking into consideration its variants and their prognosis.

2. Characteristics of pilocytic astrocytomas (PA)

PAs are the most common paediatric tumours of the central nervous system (CNS) accounting for 5% of all gliomas and 15-17% of all children and adolescent brain tumours (between 0 and 19 years), whereas they are rare in adults (about 2% of all adult brain tumours) (2). The incidence rate is 0.91 cases per 100.000 population, being highest in young children but decreasing with advancing age (2). PAs are well-circumscribed, slow-growing and low-grade astrocytic neoplasms that can arise throughout the neuroaxis but are most common in the cerebellum (42%), followed by supratentorial sites (36%), optic pathway and hypothalamus (9%), brainstem (9%) and spinal cord (2%) (2,3). Despite the cerebellar site being most common in the paediatric population, there is no difference between the cerebellar and supratentorial localisation in adults (3). The majority of PA cases are sporadic, but they are often associated with neurodevelopmental disorders with germline mutations in the Mitogen-activated protein kinase (MAPK) pathway, such as Neurofibromatosis type-1 (NF1), Noonan syndrome and encephalocraniocutaneous lipomatosis (2,4-6).

3. Clinical features

The signs and symptoms of PA are usually due to mass effect and ventricular obstruction and are strictly related to anatomical localisation. In detail, cerebellar PAs are generally characterised by symptoms due to loss of balance and coordination such as ataxia, dizziness and gait instability (7). Other symptoms have been reported due to the development of hydrocephalus and increased intracranial pressure such as headache and vomiting (7). On the other hand, PAs of the optic pathway can cause visual loss, strabismus and protrusion of the eyeball (7), whereas the hypothalamic localisation can present with hypothalamic/pituitary dysfunctions, such as obesity and diabetes insipidus (2); in addition, patients may have associated emaciation, failure to thrive and poor clinical outcome compared to PAs in other sites. Finally, spinal PAs are associated with back pain, paresis and kyphoscoliosis (2).

Paediatric PAs are frequently subtentorial and affect the cerebellum with cystic, circumscribed and indolent lesions (Fig. 1A and B). However, adult PAs are uncommon, but supratentorial lobar (mainly temporal or parietal regions) are the most frequent sites (Fig. 2A and B). In addition, similar to paediatric cases (8) in terms of survival and neurological function, adult PAs have been reported to have quite a benign course. Nevertheless, a recurrence incidence of 30% has been reported in adult PAs with a possible malignant aggressive transformation (9).

4. Imaging

The majority of PAs can be identified on magnetic resonance imaging (MRI) as well-circumscribed oval-shaped lesions with cystic components and contrast-enhancing mural nodules (Figs. 1 and 2). The solid component of PA is typically iso to hypointense on T1 imaging and hyperintense on T2 (10). Moreover, vasogenic oedema can be present, but it is less conspicuous than in higher-grade tumours, as well as non-specific calcifications (7). PAs of the optic pathways usually form fusiform masses, are accompanied by enlargement of the optic tract and are mostly associated with NF1 patients (3,11). An exophytic growth of PA is encountered when the tumour is localised in the posterior fossa, especially in the brainstem (3).

5. Pathological findings

PAs are slow-growing soft grey tissue, that characteristically occur in association with a cyst in the wall of which the tumour forms a small mural nodule, typically in the cerebellum (2). Sometimes, associated cysts may also occur in the spinal cord, basal ganglia or cerebral hemispheres (2). On smears, intraoperative examination shows cells with round to spindle nuclei with characteristic long 'hairlike' processes, Rosenthal fibres and eosinophilic granular bodies. Hemosiderin deposits, calcifications or spread in the subarachnoid space and occasional necrosis may be commonly present (2). On histopathological examination, PAs exhibit a low-to-moderate cellularity characterised by a biphasic architectural pattern, in which compact and microcystic areas are intermingled. Microscopically, densely fibrillated areas composed of elongated cells (piloid cells) with bipolar 'hairlike' processes (Fig. 3A) and bland nuclei rich in Rosenthal fibres (Fig. 3B) alternate with loose areas (Fig. 3C), with a varied degree of myxoid component composed of multipolar oligodendrocyte-like cells with round nuclei and short cytoplasmatic extension. Multinucleated cells with a 'pennies-on-a-plate' appearance may be present, as well as eosinophilic granular bodies. Mitotic activity is usually very rare (3,12). A variable amount of inflammatory cells may be encountered in PAs, mainly represented by T cells, although the role of CD4+ and CD8+ remains unclear. However, the average ratio of CD8+/CD4+ cells in PA has been reported as significantly higher than that in normal tissue, in which CD8+ and CD4+ cells were equally present (13). The higher CD8+/CD4+ ratio in PA may suggest a preferential recruitment of CD8 T cells to the tumour microenvironment, rather than a nonspecific migration of CD8 and CD4 T cells from adjacent normal brain (13). On the other hand, microglia/macrophages increase in low-grade gliomas (LGG), especially in PAs without clustering around vessels (14). Vascular proliferation with glomeruloid features and hyalinised vessels, as well as necrosis without pseudopalisading, can occur, but differential diagnoses with high-grade gliomas must be ruled out (2). Three different histological patterns of PAs can be recognised: 1) a biphasic pattern, which is the most common; 2) a predominantly compact pattern, mainly composed of pilocytic cells and Rosenthal fibres, more common in adult patients and 3) a predominantly loose pattern composed by multipolar cells, mimicking an oligodendroglioma, often associated with Fibroblast Growth Factor Receptor-1 (FGFR1) alterations (2). Microscopic infiltration of leptomeninges can be seen, especially in cerebellar and optic pathways PA. In particular, in the case of PA of the optic nerve,

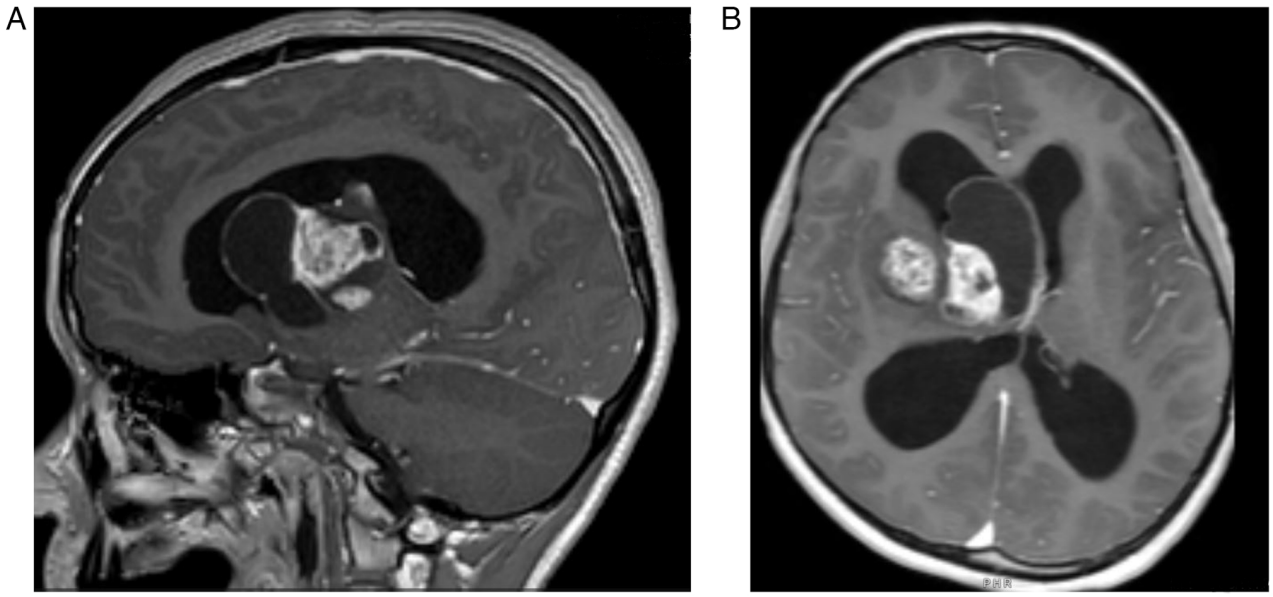

Figure 1. Case of a 13-year-old girl with a 2-week history of frontal headache, vomiting and three episodes of loss of consciousness. (A) MRI demonstrated the presence of a multiloculated, cystic, partially calcified left thalamic lesion, (B) extending into the lateral and the third ventricle, with a severe triventricular hydrocephalus.

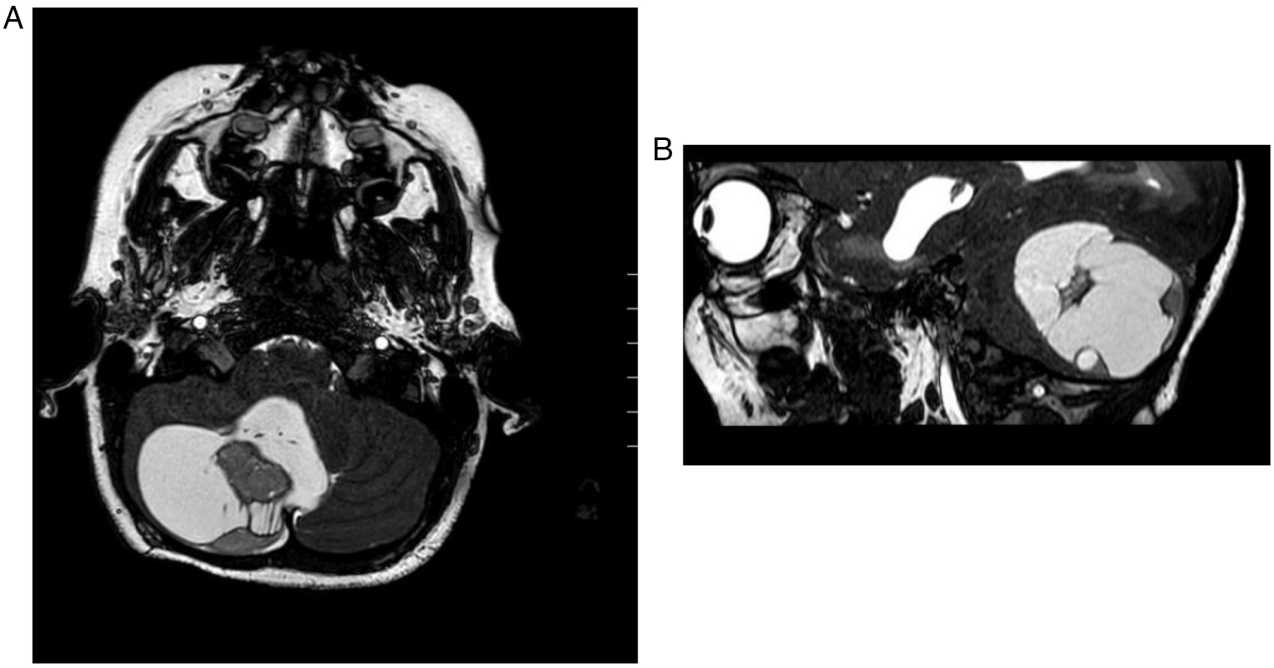

Figure 2. Case of a 6-year-old girl with a long history of headache and morning vomiting, associated with blurred vision and convergent strabismus. (A) Cross-section and (B) sagittal antero-posterior brain MRI showed a voluminous lesion, extensively occupying the vermis and predominantly the right cerebellar hemisphere with a cystic, multi-septal component converging to a solid nodule.

the tumour grows in the subarachnoid space between the nerve and the dural sheath (2,3). Immunohistochemical analysis of PAs show a strong and diffuse positivity for GFAP (Fig. 4A), S100, OLIG2 (Fig. 4B), SOX10 and p16, mainly in the classical pilocytic cells, while microcystic areas are only weakly GFAP immunoreactive (2,12). The majority of cases are positive for synaptophysin and negative for NFP, NeuN, chromogranin and CD34, although the expression of the last has been reported in some hypothalamic cases. IDH1 (R132H) (Fig. 4C) and H3K27M are not expressed. The proliferative index of Ki-67 is usually very low, less than 1% (2) (Fig. 4D).

6. Differential diagnosis of PA

Firstly, two different PA subtypes must be recognised: pilomyxoid astrocytoma (PMA) and pilocytic astrocytoma with histological features of anaplasia. PMA occurs predominantly in children, in the hypothalamic region, the suprasellar region

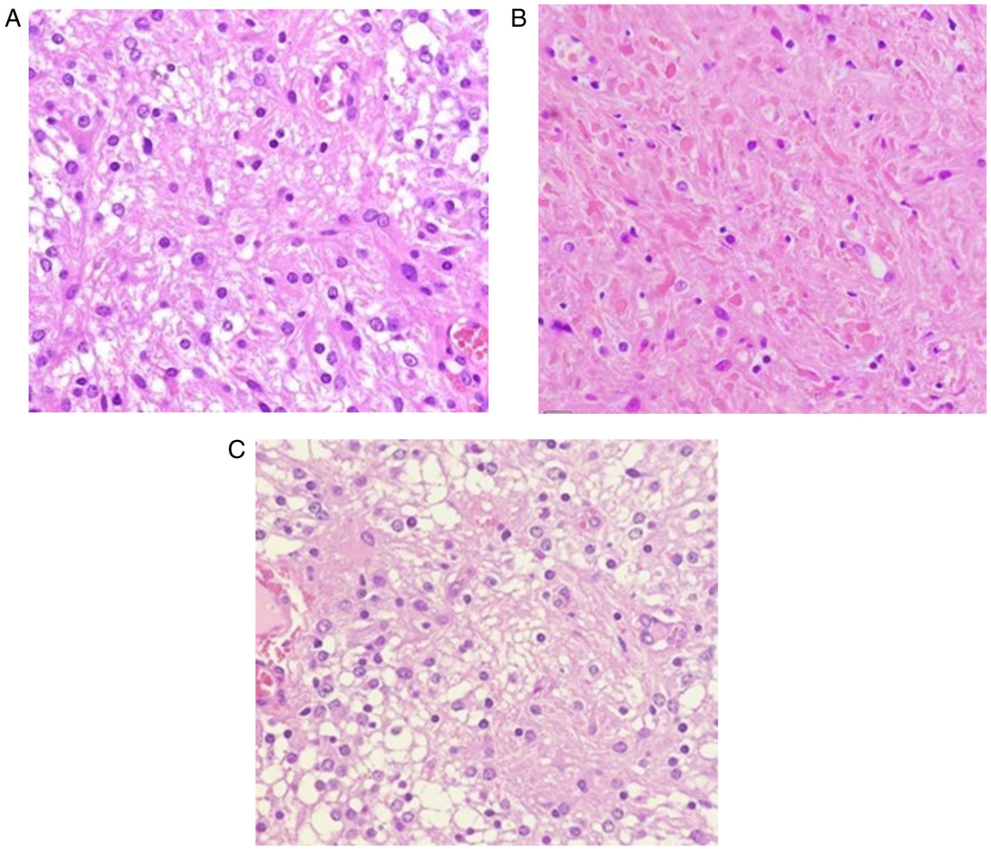

Figure 3. Morphological appearance of pilocytic astrocytomas for the case presented in Fig. 1. (A) Compact areas of bipolar cells with (B) 'hairlike' processes and (C) Rosenthal fibres alternate with loose areas with multipolar oligodendrocyte-like cells (magnification, x400; haematoxylin-eosin stain).

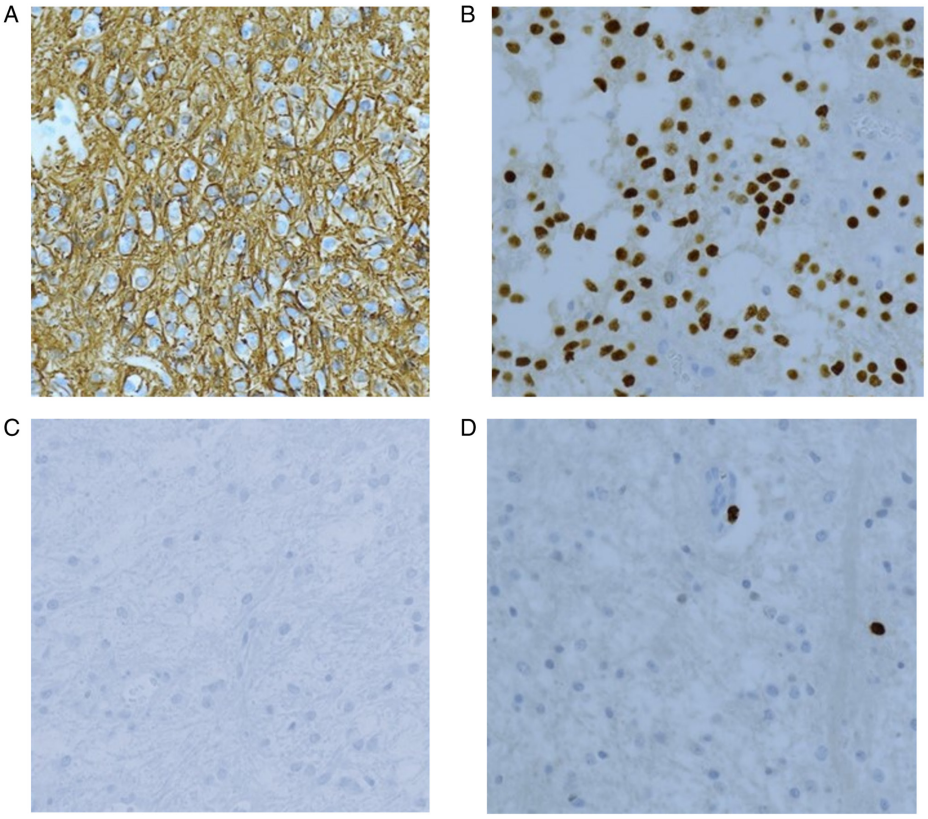

Figure 4. Immunohistochemical findings for the case presented in Fig. 1. Strong and diffuse expression of (A) glial fibrillary acidic protein and (B) oligodendrocyte transcription factor 2 documented in pilocytic astrocytomas. (C) Absence of isocitrate dehydrogenase 1 (R132) mutation. (D) KI67 proliferative index was low (magnification, x400; Mayer's nuclear counterstain).

Location	Mutation
Cerebellum (42%) 	-KIAA1549-BRAF fusion (60%) -NFI mutations -FGFR mutations -BRAF V600E mutation
Supratentorial (36%) 	-BRAF V600E mutation (5–10%) -FGFR mutations -KIAA1549-BRAF fusion
Optic pathway/ hypothalamus (9%) 	-NFI mutations (10–15%) -FGFR mutations -KIAA1549-BRAF fusion
Brainstem/midline (9%) 	-FGFR1 mutations (<5%) -KIAA1549-BRAF fusion -NFI mutations

Figure 5. Summary of the most frequent locations and molecular alterations in pilocytic astrocytomas. FGFR, fibroblast growth factor receptor; NF1, neurofibromatosis type-1.

being the most common location (67%) and shows an aggressive clinical course with a high rate of recurrence, poor clinical outcome and frequent cerebrospinal dissemination. On MRI, these tumours appear more solid and uniformly enhancing compared with typical PA. They are histologically characterised by increased cellularity composed of piloid cells admixed in abundant myxoid background; this variant typically lacks Rosenthal fibres and eosinophilic granular bodies. Therefore, there is sufficient evidence to support the classification of PMA as a more aggressive and higher grade variation of PA (15); based on imaging findings, it is really difficult to differentiate PA and PMA (16,17). The most prominent imaging characteristic of PMA is represented by intratumoural haemorrhage, which is much less common in PA. In addition, PMA shows a higher recurrence rate and often prominent cerebral-spinal fluid dissemination (18). Moreover, PA can be distinguished from pleomorphic xanthoastrocytoma (PXA), an uncommon, benign tumour in the brain that most likely develops from astrocytes, but presents with pleomorphic, xanthomatous cells (2,19). Additionally, PXA is most commonly found in the cerebral hemisphere and the leptomeninges; it occasionally occurs in the spinal cord, rarely evolving into a more malignant tumour (20). However, PA differs from dysembryoplastic neuroepithelial tumours by the absence of mucin-rich nodules and microcysts with floating neurons (2,21). Finally, the diffuse leptomeningeal glioneuronal tumour (DLGNT), a rare entity typically present in children, sometimes may present with focal areas of pilocytic features (2). Nevertheless, either 1p/19q codeletion or isolated 1p deletion with mitogen-activated protein kinase (MAPK) activating alterations may be considered characteristics in DLGNT (2). This latter finding together with the absence of isocitrate dehydrogenase (*IDH*) mutations may suggest a peculiar molecular profile of DLGNT (2).

In the differential diagnoses of PA, some additional low- and high-grade glial tumours must be considered (2,3). In particular, low-grade diffuse gliomas, such as IDH-mutant astrocytoma and oligodendroglioma, are diffusely infiltrative without Rosenthal fibres and eosinophilic granular bodies (2,3). On the other hand, the distinction from high-grade gliomas can be challenging only when PA presents high-grade histological features, such as vascular proliferation and/or necrosis, but a solid growth pattern, the presence of bipolar cells and Rosenthal fibres and low mitotic activity can be helpful in these cases (2,3)

7. Molecular features

PAs are correlated with a wide range of molecular alterations, mostly affecting the MAPK pathway (Fig. 5) (22). The most common alteration is a rearrangement of the BRAF gene, resulting in the gene fusion KIAA1549-BRAF (22,23). Other alterations of the MAPK pathway include NF1 mutations, BRAFV600E mutations, other types of BRAF fusions, KRAS mutations, FGFR1 mutations of fusions, NTRK family receptor tyrosine kinase fusions and RAF1 gene fusions. Moreover, a recent study showed that specific miRNAs, such as miR-155, miR-34a and miR-503, target genes that are involved in the regulation of the MAPK pathway in PA (24).

The KIAA1549-BRAF fusion is the most common molecular alteration, especially in cerebellar PA, accounting for 60% of all cases (2,22,23). The fusion is characterised by the tandem duplication at location 7q34, resulting in the replacement of the N-terminal end of various KIAA1549 protein exons with the N-terminal regulatory region of BRAF, while the retained BRAF kinase domain becomes unregulated and constitutively active. The most common fusion is between exon 16 of KIAA1549 and exon 9 of BRAF, followed by fusion of 15-9 exons and 16-11 exons, and then by other rare exon combinations (22). KIAA1549-BRAF fusion is recognised as a diagnostic marker in paediatric PA, whereas it is rare in adults; however, the presence of this alteration has been reported in some cases of oligodendroglioma IDH-mutated, 1p19q codeleted (25,26). Other BRAF fusions account for less than 5% (2). Several gene partners for BRAF fusions have been found in small numbers of cases, including FAM131B, RNF130, CLCN6, MKRN1, GNA11, QKI, FZR1, MACF1, GTF2I and recently GNAI3, all resulting in the loss of the N-terminal region of BRAF and the retention of the kinase domain (27-33).

NF1 somatic mutations or loss of a single wild-type allele accounts for 10-15% of all cases and are generally found in optic pathways tumours in patients with NF1 syndrome (2). Up to 33% of NF1 patients have a PA with anaplastic features and an aggressive clinical course (34).

BRAF V600E mutation accounts for 5-10% of all cases and is mostly found in supratentorial tumours and other glial and glioneuronal tumours, such as gangliogliomas and pleomorphic xanthoastrocytoma (2). It consists of the replacement of valine with glutamic acid at position 600 of the BRAF gene. BRAF V600E is the third most common mutation in PAs after KIAA1549-BRAF fusion, but the two alterations are rarely present at the same time.

FGFR1 hotspot point mutations (p.N546K, p.K656E), FGFR1-TACC1 fusion and FGFR1-internal tandem duplication (FGFR1-ITD) account for less than 5% of all cases and are mainly found in midline/brainstem tumours (2,35-37).

FGFR1 mutations are also reported in up to 60% of other glial tumours, such as dysembryoplastic neuroepithelial tumours (38). The frequency of FGFR1 mutations increases with the age of the patients and is associated with higher prevalence in sporadic optic pathway PAs in adults (39). Extremely rare molecular alterations were also identified, such as NTRK fusion with several different types of partners resulting in a constitutive dimerization and kinase activation (36), RAF1-STAGP3 fusion and RAF1-NFIA fusion resulting in constitutive RAF-1 kinase activity that leads to MEK 1/2 activation and increased cancer cell proliferation (40,41), KRAS mutations (42) and ROS1 fusions (43). Furthermore, PAs may also exhibit H3 p.K27M mutation as an exception to the rule; by contrast, diffuse midline gliomas behave aggressively when the H3 K27M mutation is documented (44,45). Therefore, taking into consideration this latter unusual molecular finding in PA, the histone H3 K27M mutation should not be considered an exclusive criterion for the diagnosis of high-grade gliomas.

On a molecular level, pilomyxoid astrocytomas harbour MAPK pathway alterations similar to typical PA, in particular, the presence of fusion gene KIAA1549-BRAF. Furthermore, a recent study revealed additional alterations in retinoic acid-mediated apoptosis and MAPK signalling pathways and key hub genes that may potentially be involved in tumour growth and progression, including BRAF, LUC7L2, MKRN1, RICTOR, TP53, HIPK2, HNF4A, POU5F and SOX4 (2,46). A distinct methylation signature called 'DNA methylation class anaplastic astrocytoma with piloid features' is encountered in pilocytic astrocytoma with unusual histological anaplastic changes (47). This latter variant is characterised by hypercellularity, cytologic atypia with or without necrosis and brisk mitotic activity (at least 5 mitoses per 10 high power fields) in an otherwise well-circumscribed, non-infiltrative lesion at the initial or recurrent diagnosis (2). Molecular features of these tumours are NF1 mutations (33%), BRAF duplications (30%), loss of ALT (69%) and ATRX expression (57%) and an alternative lengthening of telomeres phenotype (69%), the latter associated with worse overall survival (13 months) together with the presence of necrosis and anaplasia (2,29).

8. Treatment and prognosis

The treatment recommendations were based on outcomes studies relating to patient age, tumour location, surgical treatment and eventually radiation therapy. The gold standard of treatment is represented by surgical excision with complete margin resection, achieving minimal neurological damage. In fact, the complete resection has been considered the curative procedure for PA. In cystic lesions, the resection of only the nodule is recommended, but not the cyst wall (48); nevertheless, an increased thickness of the cystic wall suggests en-bloc removal of the neoplastic lesion (48,49). In detail, for subtentorial localisation of PA, the lesion should be treated through a telovelar suboccipital approach. At surgery, a clear cleavage plane may be identified for a favourable complete removal. Frequently, a serum-blood thin-walled cyst causing significant compression to the cerebellar structures may be promptly revealed. If greyish richly vascularised mural nodules were found, they should be removed. On the other hand, in the occurrence of supratentorial PAs, commonly a frontal transcortical transventricular surgical approach has been chosen. Following a frontal craniotomy, using a transnuchal corridor, the involved frontal horn of the lateral ventricle has been reached, where a voluminous thalamic lesion characterised by a thin-wall, transparent, vascularized, soft, pink cystic mass is to be removed. Conventional radiotherapy is not required; the more appropriate treatment seems to be serial follow-up, since radiotherapy frequently may carry significant sequelae. Nevertheless, if the tumour cannot be completely surgically removed due to its location, adults and older children may be subjected to radiation therapy to destroy any remaining neoplastic elements. Finally, following surgery, chemotherapy should be applied in younger children to avoid the risk of long-term growth and developmental issues. If PA recurs, a new surgical approach should be performed. It has been reported that stereotactic radiosurgery may achieve good results for residual and recurrent PA (50).

PAs are associated with a favourable prognosis and overall survival (90% in 10 years for paediatric patients, 70% in adults) (7), depending on the tumour location and resection, clinical manifestations and age of the patient. KIAAA1549-BRAF fusion has been associated with improved progression-free survival (PFS) and overall better prognosis in paediatric PA (51,52). Hypothalamic and optic pathway location has less favourable progression-free and overall survival due to an often incomplete surgical resection (53). In addition, pilomyxoid astrocytomas, pilocytic astrocytomas with histological features of anaplasia, and PA with leptomeningeal dissemination show aggressive behaviour and poorer overall survival (7,29).

Total resection is associated with better overall survival (29,54), whereas adjuvant radiotherapy, although associated with excellent progression-free survival (PFS) and overall survival (OS) (71-90 and 92-100% respectively, in 5 years), shows long-term side effects, such as second malignancies, suggesting that malignant transformation of PA occurs mainly in tumours treated with prior irradiation (54). Standard treatment with chemotherapy has been considered in the treatment of patients with low-grade gliomas and showed a 5-year PFS between 35 and 45% (54,55). These protocols comprehend the association of carboplatin with vincristine, monotherapy with vinblastine or a combination of thioguanine, procarbazine, lomustine and vincristine (54). Temozolomide has been reported not to be effective (29).

Novel therapies involving agents targeting MAPK signalling pathway dysregulation are currently in development. The most studied target agents are BRAF and MEK inhibitors (54,55). A phase II study with a MEK-inhibitor, selumetinib, in relapsed and refractory low-grade gliomas in paediatric patients showed benefits in patients with hypothalamic/optic pathway gliomas with a 24% portal response and 56% of patients with prolonged stable disease (56). Moreover, the treatment of progressive paediatric low-grade glioma with trametinib has demonstrated benefits, including 100% disease control in 18 paediatric patients (57). A combination of MEK inhibitors and chemotherapy agents such as vinblastine is currently under evaluation. Clinical trials with early-generation BRAF inhibitors (Vemurafenib, Dabrafenib, Encorafenib) are evaluating their effects on paediatric low-grade gliomas compared to standard chemotherapy treatment. Dabrafenib

plus trametinib showed clinically meaningful activity in patients with BRAFV600E-mutated recurrent or refractory high- and low-grade gliomas (58). Moreover, the same combination of BRAF inhibitors showed an excellent overall response rate (ORR) (47%) compared to carboplatin plus vincristine (11%) (59). Other studies comparing the effect of target therapy vs. standard treatment, such as selumetinib vs. carboplatin plus vincristine (NCT03871257) or trametinib vs. carboplatin plus vincristine in the Phase III LOGGIC trial, are currently ongoing, as well as the use of next-generation dimer inhibitors, such as PLX8394 and TAK580, that can potentially prevent RAF dimer formation and block MAPK activation (29). Moreover, mTOR inhibitors are also under investigation for the treatment of LGGS as monotherapy and/or in combination with other agents. In a phase II study, Everolimus showed a 2-year PFS of 39 and OS 93% in patients with relapsed LGGs (60). Additionally, Bevacizumab showed beneficial responses in a series of small studies of adult patients with unresectable PA (61,62). Finally, immune checkpoint blockade using PD-L1 or CTLA4 inhibitors may actually not be considered a potential therapeutic choice for unresectable or recurrent PA since a low positive rate, as well as a low percentage of positive neoplastic or immune cells, has been documented (63). However, the amount of infiltrating T cells in PA is variable, although the contribution of $CD4^+$ and $CD8^+$ T cells remains unclear. In detail, higher levels of T cell infiltration have been encountered in LGG in comparison to their malignant counterparts, suggesting that $CD8^+$ T cell content is related to improved patient survival (64). Moreover, the intriguing role of T cells in LGG growth has been also revealed by sex-specific differences in T-cell content in supratentorial vs. infratentorial PA (65). The additional lack of tumoral PD-1/PD-L1 immunoexpression strongly suggests the need for further investigations to better define immune cell-directed targets in PA (65).

9. Conclusions and future perspectives

The recent updating of the WHO Classification of CNS tumours has focused on the need to integrate molecular information into the neuropathological profile of brain tumours, including PA. Since molecular findings appear complex, the attempts to utilise specific individualised treatment for PA will need the results of ongoing trials targeting the MAP kinase pathway. Future insights including morphological, molecular and epigenetic characteristics of PA are mandatory to approach a personalised treatment, also taking into consideration the low rate of PA incidence in children and adults, which requires a multi-institution team or the institution of a specific registry to determine the optimal targeted agents. In this direction, the application of different methodologies, such as fluorescent *in situ* hybridisation, polymerase chain reaction (PCR) and reverse transcription-PCR, may be helpful to better define characteristic molecular alterations in PA, driving the choice of an individual oncological treatment, even in the event of recurrence. In particular, molecular targets are continually expanding, including novel BRAF and RAF gene fusions such as FAM131-BRAF, SRGAP3-RAF1, RNF130-BRAF, CLCN6 -BRAF, MKRN1-BRAF and GNAI1-BRAF, which may represent an important area for continued investigation to better characterise their therapeutic and prognostic significance.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

CP, AI and GT contributed to conception, design and the draft of the manuscript. CP, VF and AG were involved in acquisition and interpretation of data. MM, AI and GT critically reviewed/edited the manuscript. AG and GT confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethical approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Local Bioethical Committee at the University Hospital 'G. Martino' of Messina (Messina, Italy) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The present study was submitted to The Institutional Review Board of the University Hospital of Messina (Messina, Italy) to discuss and approve the study (prot. 47/19; May 2, 2019). Written anonymized informed consent was obtained from all patients; for children, the consent was signed by the parents/guardians.

Patient consent for publication

Written informed consent for the publication of their data was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

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