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ORIGINAL ARTICLE – CANCER RESEARCH

Gliomas in children and adolescents: investigation of molecular alterations with a potential prognostic and therapeutic impact

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Abstract

Purpose Gliomas represent the most frequent central nervous system (CNS) tumors in children and adolescents. However, therapeutic strategies for these patients, based on tumor molecular profile, are still limited compared to the wide range of treatment options for the adult population. We investigated molecular alterations, with a potential prognostic marker and therapeutic target in gliomas of childhood and adolescence using the next-generation sequencing (NGS) strategy.

Methods We selected 95 samples with initial diagnosis of glioma from patients treated at Pediatric Oncology Institute-GRAACC/UNIFESP. All samples were categorized according to the 2021 World Health Organization Classification of Tumors of the CNS, which included 39 low-grade gliomas (LGGs) and 56 high-grade gliomas (HGGs). Four HGG samples were classified as congenital glioblastoma (cGBM). NGS was performed to identify somatic genetic variants in tumor samples using the Oncomine Childhood Cancer Research Assay® (OCCRA®) panel, from Thermo Fisher Scientific®.

Results Genetic variants were identified in 76 of 95 (80%) tumors. In HGGs, the most common molecular alteration detected was *H3F3A* c.83A > T variant (H3.3 K27M) and co-occurring mutations in *ATRX, TP53, PDGFRA, MET*, and *MYC* genes were also frequently observed. One HGG sample was reclassified as supratentorial ependymoma *ZFTA*-fusion positive after NGS was performed. In LGGs, four *KIAA1549–BRAF* fusion transcripts were detected and this alteration was the most recurrent genetic event and favorable prognostic factor identified. Additionally, genetic variants in *ALK* and *NTRK* genes, which provide potential targets for therapy with Food and Drug Administration-approved drugs, were identified in two different cases of cGBM that were classified as infant-type hemispheric glioma, a newly recognized subgroup of pediatric HGG.

Conclusion Molecular profiling by the OCCRA® panel comprehensively addressed the most relevant genetic variants in gliomas of childhood and adolescence, as these tumors have specific patterns of molecular alterations, outcomes, and effectiveness to therapies.

Keywords Gliomas · Central nervous system tumor · Pediatric brain tumor · Congenital glioblastoma · Next-generation sequency · Molecular profiling

Introduction

The fifth edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS), published in 2021, introduces major changes involving the classification of gliomas, as this tumor is separated into pediatric-type and adult-type, given their well-established molecular and genetic differences. In this context, gliomas occurring in children can be mainly categorized as low-grade and high-grade gliomas (Louis et al. 2021; Wen and Packer 2021).

Low-grade gliomas (LGGs) are a diverse spectrum of grade 1 and 2 tumors that represent the most common CNS neoplasms in children, accounting for approximately 30–40% of all primary pediatric brain tumors (Hargrave 2009). Pilocytic astrocytoma (PA) is the most frequent histological subtype of circumscribed astrocytic glioma in children and adolescents, and these patients frequently display an overall survival (OS) rate that can exceed 90% in 10 years. However, local recurrence occurs in 10–20%

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of cases and in patients where the primary lesion was not subjected to gross total resection, tumor progression rate is about 50–80% (Hargrave 2009; Jones et al. 2012). In contrast to adult LGGs, alterations in *IDH* gene are extremely rare in children, with the gain of 7q34 region of *BRAF* oncogene being the commonest mutation in childhood tumors (Drobysheva et al. 2017).

High-grade gliomas (HGGs) are malignant, diffuse, and infiltrating grade 3 and 4 tumors that represent 8–12% of all childhood CNS neoplasms. Pediatric patients with HGG show long-term OS rates lower than 10% and in most cases, these tumors are incurable. Childhood HGGs can be classified into subgroups based on their most recurrent genetic and epigenetic alterations (Braunstein et al. 2017; Fangusaro 2012). Somatic histone H3 alterations, such as K27M and G34R/V mutations, are specific molecular markers for pediatric HGGs. Recent studies have identified K27M mutation in *H3F3A* and *HIST1H3B* genes, responsible for encoding the H3.3 and H3.1 histone variants, respectively. Pediatric HGGs with *H3F3A* and *HIST1H3B* K27M alterations are midline tumors frequently observed in children that show a poor prognosis, while *H3F3A* G34R/V mutations occur in hemispheric tumors, are commonly identified in adolescents and young adults, and are associated with a slightly longer survival. Accordingly, these tumors comprise two main subgroups of pediatric HGG denoted as diffuse midline glioma H3 K27-altered and diffuse hemispheric glioma H3 G34-mutant (Ebrahimi et al. 2019; Sturm et al. 2014; Louis et al. 2021). Genome-wide methylation profiling of childhood HGG recently revealed six epigenetically distinct subgroups with alterations in *H3F3A* (H3.3 K27M and H3.3 G34R/V), *HIST1H3B* (H3.1 K27M), *IDH1/2* (*IDH*), and *BRAF* (*BRAF* V600E) genes (Pollack, Agnihotri, and Broniscer 2019).

Congenital brain tumors, defined by presentation prenatally or within the first months of life, represent approximately 2% of all pediatric CNS tumors, with congenital glioblastoma (cGBM) being among the rarest type (Solitare and Krigman 1964; Severino et al. 2010; Macy et al. 2012). To date, the most frequently genetic alterations identified in cGBM are *ALK* and *ROS1* gene fusions. Thus, recent studies have proposed that cGBM is genetically distinct from HGG that affects older children and adults, as these tumors harbor a unique molecular profile (Gilani et al. 2020; Macy et al. 2012). These findings suggest that cGBM may comprises a specific entity of infant-type hemispheric glioma, a novel tumor type recognized by the 2021 WHO Classification of Tumors of the CNS, characterized by newborns and infants that show fusions in *ALK, ROS1, NTRK,* or *MET* genes (Louis et al. 2021).

Therapeutic strategies for pediatric neoplasms, based on tumor molecular profile, are still limited compared to the wide range of treatment options for the adult population (Ahmed et al. 2018; Lorentzian et al. 2018). The Oncomine Childhood Cancer Research Assay® (OCCRA®) is the first next-generation sequencing (NGS) panel designed exclusively for the investigation of the main genetic events of childhood and adolescence neoplasms (Hiemenz et al. 2018). Considering the genomic and prognostic heterogeneity of pediatric and adolescent gliomas, as well as the unavailability of agents that target the most common molecular alterations present in these tumors, a specific NGS genetic panel can provide information for patients' stratification into risk groups and possible therapeutic targets (Pollack et al. 2019; Terashima and Ogiwara 2021).

Therefore, the present study aimed to investigate molecular alterations, with a potential prognostic marker and therapeutic target in gliomas of childhood and adolescence using the NGS strategy. We identified somatic genetic variants in tumor samples for a comprehensive molecular profiling of LGGs and HGGs using the OCCRA® panel.

Materials and methods

Tumor samples

All 95 fresh-frozen tumor samples used in this study were obtained from patients treated at Pediatric Oncology Institute-Grupo de Apoio ao Adolescente e à Criança com Câncer/Federal University of Sao Paulo (IOP-GRAACC/UNIFESP), between 2000 and 2020, and belong to the Institute Biobank (National Commission of Ethics in Research—CONEP B-053). All 95 cases with initial diagnosis of glioma were identified via a retrospective database review. This study was approved by the Institutional Research Committee (Committee for Ethics in Research—Federal University of Sao Paulo no. 0915/2019) and written informed consent was obtained from all patients/guardians.

All primary tumors were classified according to the morphological characteristics of the tumor tissues analyzed by immunohistochemistry and based on the 2021 WHO Classification of Tumors of the CNS (Louis et al. 2021). All internal cases were jointly reevaluated by two or three pathologists of our Institution and inclusion criteria was histological diagnosis of WHO grade 1, 2, 3, and 4 glioma. Based on our molecular findings using NGS, one HGG sample was reclassified as supratentorial ependymoma (ST-EPN) *ZFTA*-fusion positive, and excluded from patients' clinical and survival analysis.

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DNA and RNA isolation and quantification

Total DNA and RNA were extracted from frozen tumor samples using FastPrep®-24 Tissue Ruptor (MP Biomedicals®) and AllPrep DNA/RNA Mini Kit (QIAGEN®) according to the manufacturer's instructions. After extraction, samples were quantified in Nanodrop 2000® and in Qubit 3® fluorometer using Qubit RNA HS Assay Kit® and Qubit dsDNA HS Assay Kit® (Thermo Fisher Scientific®). The ratio between the 260 nm and 280 nm absorbances was used as quality control (QC) for the extractions. An A260/280 ratio between 1.6–1.8 and 1.8–2.0 was adopted for DNA and RNA, respectively. Only samples with the expected QC standards were included in the study. After quantification, synthesis of complementary DNA (cDNA) was performed using 20 ng of total RNA according to the manufacturer's protocol of SuperScript Vilo IV® (Thermo Fisher Scientific®).

Library preparation

Sequencing libraries were prepared from 20 ng of DNA and 20 ng of cDNA from each tumor sample, following the manufacture's protocol for the OCCRA® (Thermo Fisher Scientific®). Amplicon libraries with barcodes specific to each sample were generated from DNA and cDNA, and automated clonal amplification was performed on the Ion Chef™ System (Thermo Fisher Scientific®). Only the beads that were enriched with a unique template (monoclonal), that yielded reads of 25 bp or longer after a 3′15 bp-wide sliding-window quality trimming approach with q17 threshold, and which do not belong to the library internal controls, were considered for further analyses. After the preparation was completed, the libraries were loaded in the Ion 540™ chip (Thermo Fisher Scientific®) and inserted on the Ion S5™ System Sequencer (Thermo Fisher Scientific®).

Next-generation sequencing run

Sequencing was performed using 200-bp reads, and at the end of the sequencing run, the data generated by the large-scale sequencing was performed, and the quality for each reading was evaluated using Torrent Suite™ software version 5.2.1 (Thermo Fisher Scientific®). Only amplicon readings that showed a ratio between the forward and reverse primers ≥ 0.6 and ≤ 1.4 were selected. The readings obtained were aligned to the hg19/GHCh37 human reference genome on the Torrent Suite™ software and were considered readings with a minimum coverage of 2000 ×. The generated BAM files were analyzed using Integrative Genomics Viewer software (IGV Software, San Diego, CA, USA). The Variant Call Format files were obtained and the variants identified were compared to the following databases: COSMIC, dbSNP, and ExAC.

Variant analysis using the Oncomine Childhood Cancer Research Assay® panel

All tumor samples included in this study were subjected to targeted sequencing using the OCCRA® panel that comprises a total of 255 tumor-associated genes (Table S1 Supplementary Material). Sequencing analysis contains information about somatic variants, including multi-nucleotide variants (MNVs), single-nucleotide variants (SNVs), insertions or deletions (InDels), copy number variations (CNVs), and gene fusions. Variants were classified according to class (SNV or InDel), functional effect (missense, nonsense, synonymous, frameshift, and nonframeshift), and clinical significance (pathogenic, likely pathogenic, uncertain significance, likely benign, and benign) according to FATHMM prediction scores (0–1), with score ≥ 0.7 classified as pathogenic. For CNVs, samples with MAPD ≤ 0.35 were considered in the study. Variant characteristics were evaluated using COSMIC, ClinVar, VARSOME, USCS Genome Browser, and Ensembl Genome Browser databases.

Data and statistical analysis

Data analysis was performed using IBM SPSS Statistics version 21.0 (Armonk, NY: IBM Corp), *jamovi* version 1.2 (The Jamovi Project, Sidney, AU), and GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA) for Windows. OS curves were generated by applying Kaplan–Meier method with a 95% confidence interval (95% CI), and then compared by Log-rank test. Statistical significance was taken as $p < 0.05$ (5%).

Results

Patient and tumor characteristics

Clinical features of the patients analyzed in this study are summarized in Table 1 and molecular classification can be found in Table 2. The sex ratio was 1:1 (male: 51%; female: 49%), and the median age at diagnosis was 5.8 years (range: 0.3–16.6 years) and 7.5 years (range: 0–18.7 years) for LGG and HGG patients, respectively.

LGGs (grade 1/2) were comprised by 27 (29%) cases of PA, one (1%) case of ganglioglioma, a glioneuronal tumor, five (5%) cases of diffuse LGG MAPK pathway-wildtype, and five (5%) cases of diffuse LGG MAPK pathway-altered. One (1%) case included a pilomyxoid astrocytoma (PMA), a variant of PA that exhibits a more aggressive behavior.

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Table 1 Clinical features of the patients analyzed in the study

	Total ($N=94$)			
	Low-grade gliomas		High-grade gliomas	
	$N=39$		$N=55$	
Characteristic	No.	%	No.	%
Age at diagnosis, years				
Median	5.8		7.5	
Range	0.3–16.6		0–18.7	
Mean	6.8		8.5	
Standard deviation	4.4		5.7	
Follow-up time, months				
Median	51.2		19.0	
5-year overall survival				
Rate	90.7%		24.6%	
10-year overall survival				
Rate	87.2%		12.3%	
Sex				
Female	23	59	23	42
Male	16	41	32	58
Tumor location				
Hemispheric	4	10	24	43
Diencephalic/Thalamic	1	3	13	24
Optic/Suprasellar	3	8	1	2
Ventricular/Intraventricular	2	5	3	5
Posterior Fossa	29	74	7	13
Spinal	0	0	7	13
Relapse				
Yes	6	15	11	20
No	33	85	44	80
Actual status				
Complete remission	22	56	10	18
In treatment	12	31	6	11
Dead	5	13	39	71
Treatment				
Chemotherapy	6	15	11	20
Radiotherapy	2	5	8	15
Chemotherapy + Radiotherapy	5	13	27	49
None	26	67	9	16

HGGs (grade 3/4) consisted of two (2%) cases of pleomorphic xanthoastrocytoma (PXA), one (1%) case of malignant ganglioglioma, 18 cases of H3 K27-altered gliomas, including 16 (17%) midline (diffuse midline glioma H3 K27-altered) and two (2%) hemispheric (diffuse glioma H3 K27-altered) tumors, one (1%) case of diffuse hemispheric glioma H3G34-mutant, 31 (33%) cases of diffuse HGG H3-wildtype and IDH-wildtype, and two (2%) cases of infant-type hemispheric glioma. One (1%) HGG sample was reclassified as ST-EPN *ZFTA*-fusion positive.

Table 2 Molecular classification of tumor samples analyzed in the study, according to the 2021 WHO Classification of Tumors of the CNS

Molecular classification	No.	%
Low-grade gliomas		
Pilocytic astrocytoma	27	29
Pilomyxoid astrocytoma	1	1
Diffuse low-grade glioma, MAPK pathway-wildtype	5	5
Diffuse low-grade glioma, MAPK pathway-altered	5	5
High-grade gliomas		
Pleomorphic xanthoastrocytoma	2	2
Diffuse midline glioma, H3 K27-altered	16	17
Diffuse glioma, H3 K27-altered	2	2
Diffuse hemispheric glioma, H3 G34-mutant	1	1
Diffuse high-grade glioma, H3-wildtype and IDH-wildtype	31	33
Infant-type hemispheric glioma	2	2
Glioneuronal and neuronal tumors		
Ganglioglioma	2	2
Reclassification		
Supratentorial ependymoma, *ZFTA*-fusion positive	1	1
	95	100

A total of 20 cases were infantile gliomas and four cases were classified as cGBM. Classification criteria for infant HGG was diagnosis under 5 years old (Clarke et al. 2020) and congenital cases consisted in those patients who presented clinical symptoms at birth, within the first weeks of life, or within the first 3 months of age. (Solitare and Krigman 1964). Among the four patients with cGBM analyzed, two patients had a postnatal diagnosis, with mean age at diagnosis of 1.3 months, and two patients were diagnosed during the prenatal. cGBM patients included four females and all tumors affected the cerebral hemispheres of the supratentorial compartment. Additionally, two cGBM cases were classified as infant-type hemispheric glioma, a newly recognized group of pediatric HGG (Louis et al. 2021).

Identification of genetic variants in glioma samples

A total of 95 samples with initial diagnosis of glioma were sequenced to investigate the presence of somatic genetic variants using the OCCRA© panel. Sequencing analyses showed that variants were detected in 76 of 95 (80%) tumors - 33 LGGs (43.4%) and 43 HGGs (56.6%). We observed 51 altered genes and 123 genetic variants, which included 60 SNVs (48.8%), 22 InDels (17.9%), 29 CNVs (23.6%), and 12 gene fusions (9.7%). *KIAA1549–BRAF* fusions were the most frequent events in LGGs (26/33 = 78.8%) and variants in *H3F3A* (17/43 = 39.5%), *TP53* (17/43 = 39.5%), and *ATRX* (9/43 = 20.9%) genes were responsible for the majority of all alterations identified in HGGs. The distribution

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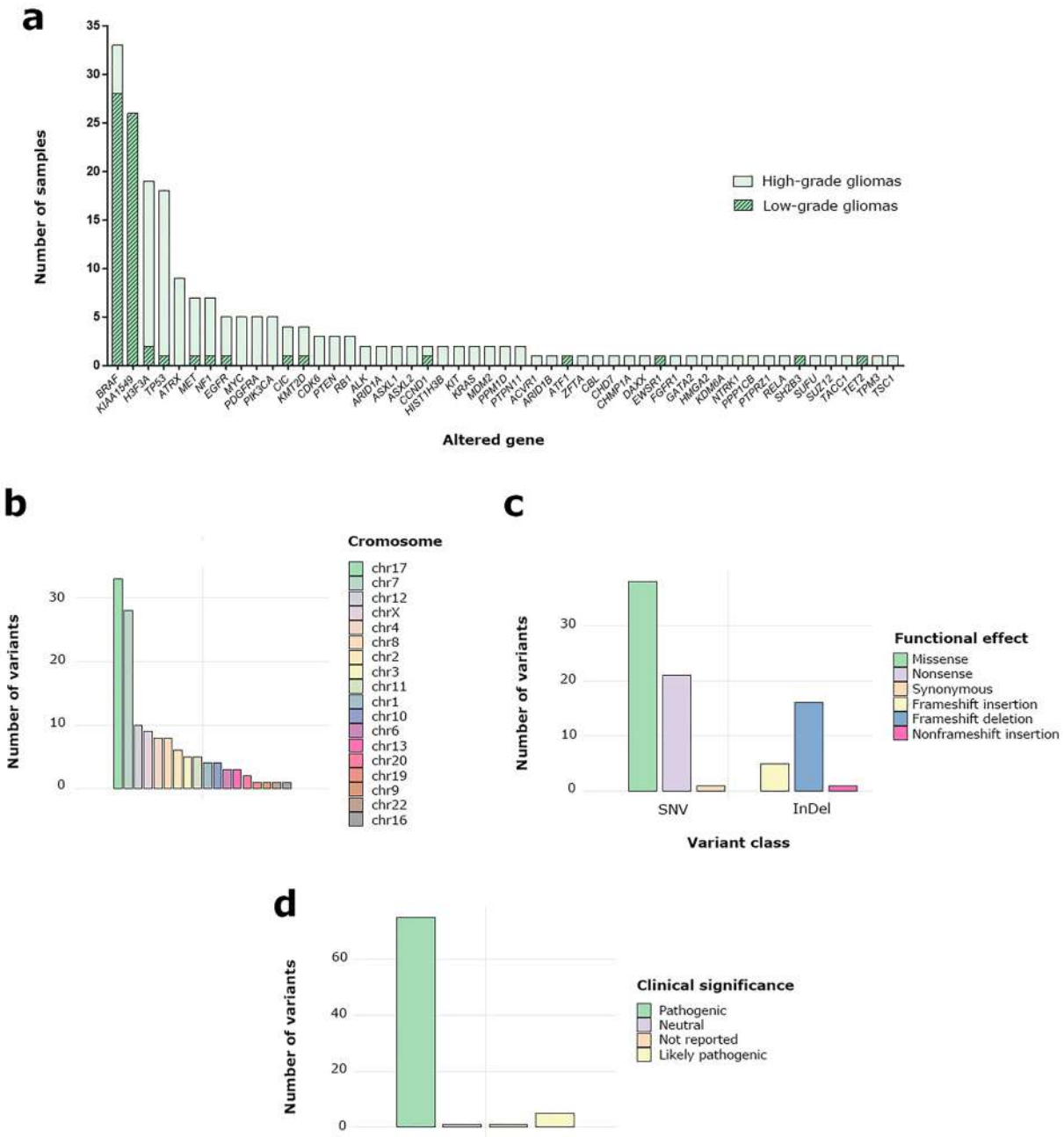

Fig. 1 Genetic alterations identified in tumor samples. **a** Frequency of mutations by gene in the 95 samples. **b** Number of variants located on each chromosome. **c** Number of variants according to class and functional effect. **d** Number of variants according to clinical significance

of altered genes in glioma samples is shown in Fig. 1a, and profile of genetic variants identified in tumor samples can be found in Table S2 (Supplementary Material).

Genetic variants analysis

Chromosome 17 had the highest number of variants (25%), followed by chromosome 7 (21.2%), as *TP53, KIAA1549,* and *BRAF* genes were the most frequently altered genes detected in tumor samples and are located at 17p13.1 and 7q34, respectively (Fig. 1b). Variants with missense and nonsense effect were the most common, accounting for 46.3% and 25.6% of cases, respectively. InDels were also frequently identified, as frameshift deletion variants were responsible for 19.6% of cases, and frameshift insertions were observed in 6.1% of cases. Synonymous and nonframeshift variants were found in 1.2% of cases each (Fig. 1c). Of all variants analyzed, 91.5% were pathogenic,

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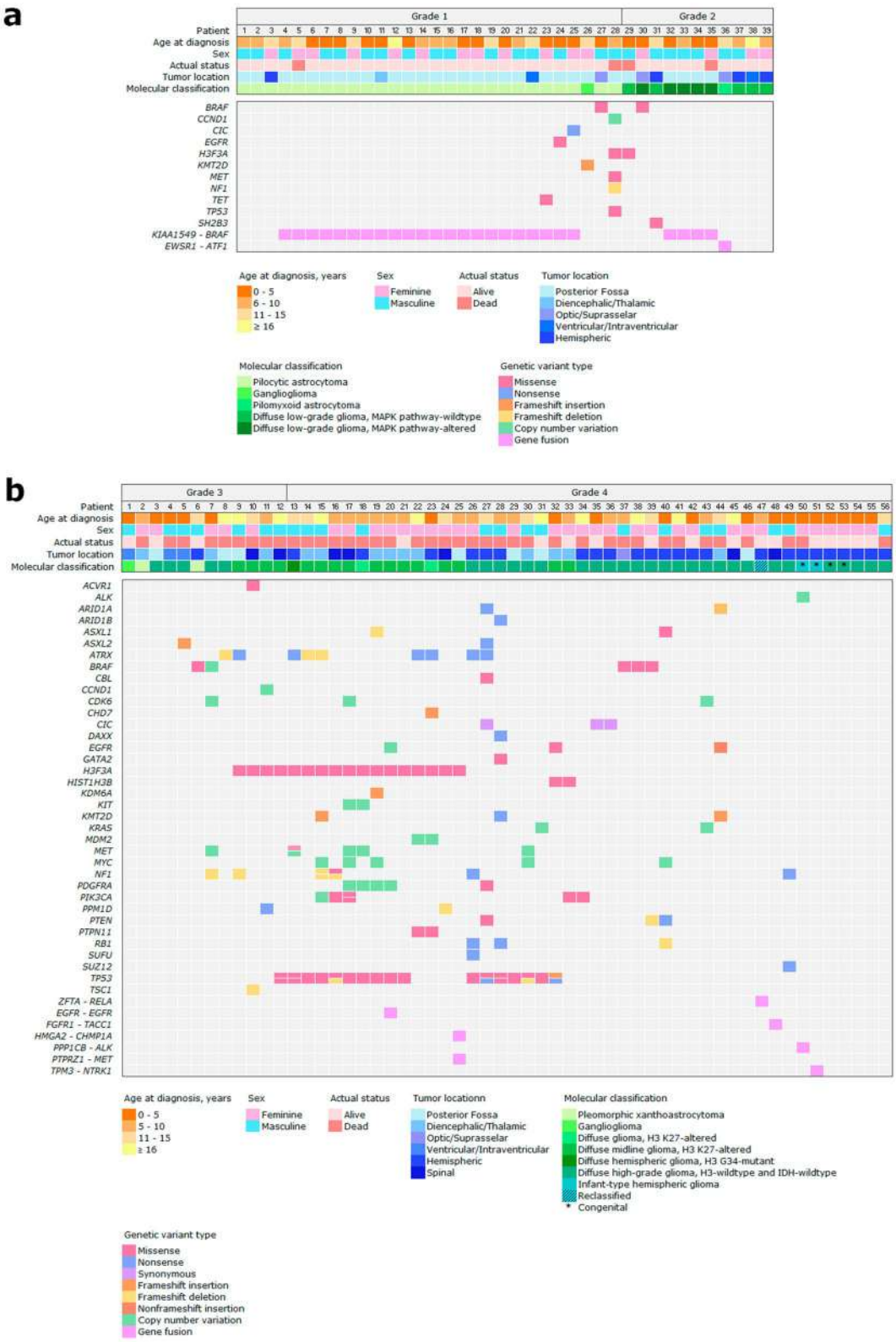

Fig. 2 Clinical and molecular profile of gliomas. **a** Genomic landscape of 39 LGG samples. **b** Genomic landscape of 56 HGG samples. Left pane indicates the altered gene across all the samples and each vertical bar represents an individual case. The presence of more than one variant in the same gene is represented by multicolored boxes

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Table 3 *KIAA1549–BRAF* fusion transcripts detected in LGG samples

Gene fusion	Gene 1			Gene 2			Total ($N = 26$)	
	Name	Chr position	Exon	Name	Chr position	Exon	No.samples	%
KIAA1549-BRAF	*KIAA1549*	7:138552721	14	*BRAF*	7:140487384	9	8	30.8
	KIAA1549	7:138545885	15	*BRAF*	7:140487385	9	11	42.3
	KIAA1549	7:138545885	15	*BRAF*	7:140481493	11	6	23.1
	KIAA1549	7:138529062	17	*BRAF*	7:140482957	10	1	3.8

Chr cromosome

6.1% were likely pathogenic, 1.2% were neutral, and 1.2% have not been reported yet (Fig. 1d).

Comprehensive molecular profiling of gliomas

The genomic landscape of LGG and HGG patients analyzed in the present study using the OCCRA® panel can be observed in Fig. 2a and b, respectively. A total of 26 *KIAA1549–BRAF* fusions were detected in LGGs, and among them, 22 (84.7%) cases were identified in PA samples. Four *KIAA1549–BRAF* fusion transcripts were detected and the most common was between exon 15 of *KIAA1549* and exon 9 of *BRAF* (15;9), observed in 11 of 26 (42.3%) cases. *KIAA1549–BRAF* fusions 14;9 and 15;11 were also prevalent, observed in eight (30.8%) and six (23.1%) of 26 cases, respectively. Less common fusion transcript included exon 17 of *KIAA1549* and exon 10 of *BRAF* (17;10), which was identified in one case (3.8%) (Table 3). In addition, a gene fusion between exon 7 of *EWSR1* and exon 5 of *ATF1* (*EWSR1-ATF1*) was detected in one PMA sample in the LGG subgroup.

In HGGs, c.83A > T (H3.3 K27M) and c.103G > A (H3.3 G34R) variants of *H3F3A* gene were identified in 16 cases and one case, respectively. *BRAF* c.1799 T > A variant (*BRAF* V600E) was observed in four cases, including one PXA. No alterations in *IDH1* or *IDH2* genes were detected by the panel. Interestingly, co-occurring alterations were observed in HGG patients harboring variants in *H3F3A* gene—58.8% (10/17) of cases had variants in *TP53*, 35.3% (6/17) of cases showed *ATRX* loss-of-function mutations, 23.5% (4/17) of cases had *PDGFRA*-amplification, and 35.2% (6/17) of cases showed *MET* or *MYC* amplifications. Moreover, three tumors had concomitantly variants in *H3F3A*, *TP53*, and *ATRX* genes (Fig. 3a). A gene fusion between exon 3 of *ZFTA* and exon 2 of *RELA* (*ZFTA-RELA*) was detected in one HGG case that was reclassified as ST-EPN *ZFTA*-fusion positive.

Of all four cGBM patients analyzed, two cases exhibited tumors with genetic variants. One patient showed an increase of 11.2 copies of *ALK* and a gene fusion between exon 5 of *PPP1CB* and exon 20 of *ALK* (*PPP1CB-ALK),* and one patient had a *NTRK* gene fusion, which consisted in exon 7 of *TPM3* and exon 10 of *NTRK1 (TPM3-NTRK1)* (Table 4). Both tumors were classified as infant-type hemispheric glioma.

Subgroup of HGG in children and adolescents with HGG subgroup with histone H3.3 mutations

H3F3A c.83A > T variant was responsible for the majority of all genetic alterations detected in HGGs. Of all 16 H3.3 K27-altered gliomas analyzed in the present study, 14 affected midline regions of the CNS, which included seven (43.8%) thalamic tumors, five (31.2%) spinal, and two (12.5%) from the cerebellum/brainstem, whereas two (12.5%) cases samples affected the cerebral hemisphere of the supratentorial region. The only H3.3 G34 mutant tumor identified was hemispheric (Fig. 3 b).

HGG patients with H3.3 K27M mutation had a median OS of 9.1 months (95% CI 4.0–14.3), and OS curves analysis showed a statistical difference between H3.3 K27-altered ($n = 16$) and H3.3 K27-wildtype ($n = 13$) subgroups ($\chi^2 = 11.99$, $p = 0.0005$). Patients with H3.3 K27M mutation had a worse 5-year OS (rate: 6.3%) in comparison to the wildtype subgroup (rate: 61.5%) (Fig. 3c).

High frequency of DNA copy number variations in HGGs

Of all 29 CNVs detected by the panel, 28 (96.5%) were identified in HGGs. Increase in the copy number of 12 genes was observed, with *MET* (5/28 = 17.8%), *MYC* (5/28 = 17.8%) and *PDGFRA* (4/28 = 14.3%) genes being responsible for the majority of cases. The median DNA copy number was 6.9 copies (range: 1.2–270 copies; standard deviation: 50.1) and the distribution of all CNVs detected in HGG samples is summarized in Table S3 (Supplementary Material).

Survival comparisons between tumor subgroups

LGG and HGG patients included in the study were observed for a median of 51.2 months and 19 months, respectively, from date of diagnosis to last contact or death. For LGG patients, the median OS was 187.2 months (95% CI

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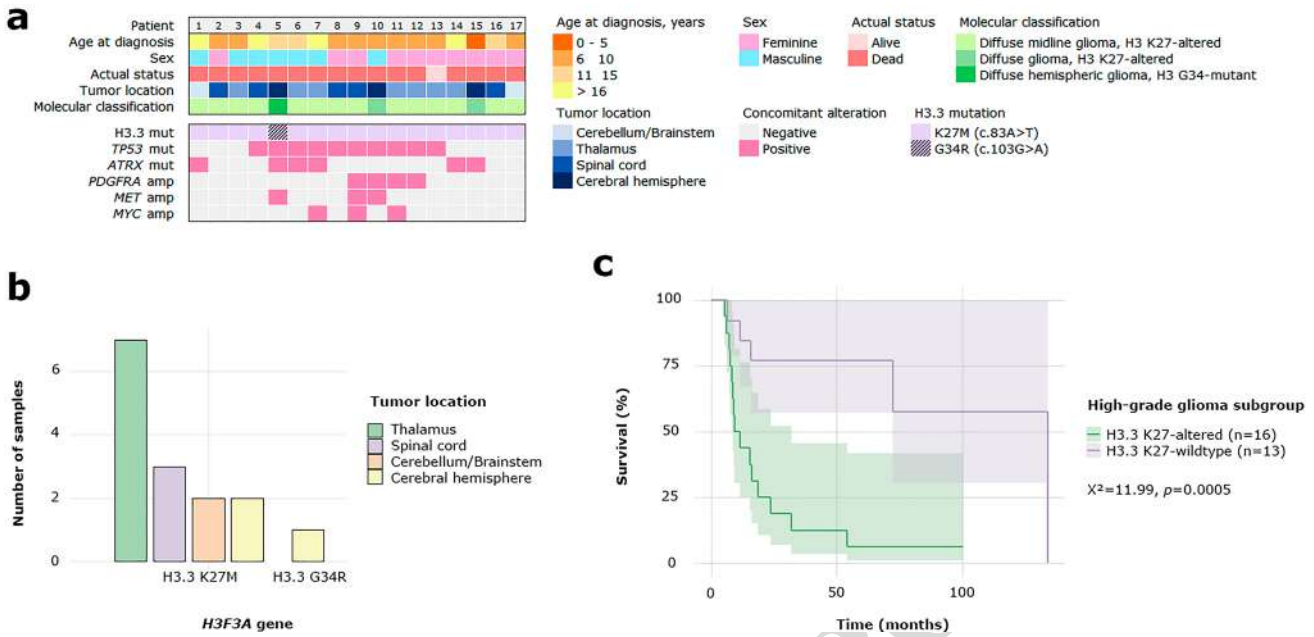

Fig. 3 Subgroup of HGG in children and adolescents with histone H3.3 alterations **a** Clinical and molecular profile of HGG patients with H3.3 K27M or G34R mutation (H3.3 K27M or H3.3 G34R) and concomitant variants involving *TP53, ATRX, PDGFRA, MET,* and *MYC* genes. **b** Number of samples with H3.3 K27M or G34R mutation, according to tumor location. **c** Patients with H3.3 K27M-altered tumors showed a worse 5-year overall survival when compared to the wildtype subgroup ($\chi^2 = 11.99$, $p = 0.0005$). *mut* mutation, *amp* amplification

182.5–191.8) and the 5-year and 10-year OS rates were 90.7% and 87.2%, respectively. For HGG patients, the median OS was 23.6 months (95% CI 10.3–36.8) and the 5-year and 10-year OS rates were 24.6% and 12.3%, respectively.

OS curves showed a statistical difference between tumor subgroups ($\chi^2 = 34.59$, $p < 0.0001$) (Fig. 4a), as HGG patients revealed a worse 10-year OS when compared to LGG patients. Also, infant patients with HGG (diagnosis < 5 years old; $n = 20$) showed a better 10-year OS (rate: 45.7%) when compared to older patients with HGG (diagnosis > 5 years old; $n = 35$) (rate: 14.9%; $\chi^2 = 4.57$, $p = 0.0324$) (Fig. 4b).

Discussion

Recent advancements in the molecular understanding of pediatric gliomas revealed that each tumor subgroup can harbor specific genetic alterations. However, the development of new targeted treatments for children and adolescents with glioma remains a major challenge (Terashima and Ogiwara 2021; Blattner-Johnson et al. 2021). Considering that gliomas in children differ on a clinical and molecular basis from gliomas that occur in adults, we aimed to investigate the presence of somatic genetic variants, with a potential prognostic and therapeutic impact in LGGs and HGGs of childhood and adolescence, for a comprehensive tumor profiling.

Mutations in *BRAF* gene represent the most frequently known and well-established genetic alterations in pediatric LGGs. *KIAA1549–BRAF* fusion is detected in 50–85% of PA, leading to deregulation of the *MAPK* signaling pathway (Jones et al. 2008; Kaul et al. 2012). Currently, there are nine known *KIAA1549–BRAF* fusion junctions, with the majority involving fusions between *KIAA1549* exon 16 and *BRAF* exon 9 (16;9) (68%), *KIAA1549* exon 16 and *BRAF* exon 11 (16;11) (10%), and *KIAA1549* exon 15 and *BRAF* exon 9 (15;9) (9%) (Srinivasa et al. 2020).

In the present study, *KIAA1549–BRAF* 15;9 was the most frequent fusion transcript detected in LGGs, accounting for 42.3% of cases, a higher rate than the described in the literature for this fusion variant. The second fusion transcriptt most detected, *KIAA1549–BRAF* 14;9 (30.8%), has not been reported in PA yet. Unexpectedly, 16;9 fusion transcript, commonly known as the most recurrent, was not identified in our tumor samples. Our clinical and molecular analysis revealed that *KIAA1549–BRAF* fusion was the most significant favorable prognostic factor in LGGs, since patients with this fusion had a 5-year OS rate of 100% and 95.7% of these patients showed no other additional alterations detected by the panel. It has been suggested that *KIAA1549–BRAF* may

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Table 4 Clinical and molecular characteristics of four patients diagnosed with cGBM

Patient	Gender	Age at diagnosis	Tumor location	CNS region	Treatment	Status	Relapse	Overall survival	Variant class	Gene
1	Female	3 days	Hemispheric	Parietal	None	Dead	No	2.1 days	CNV	*ALK*
									Gene fusion	*PPP1CB-ALK*
2	Female	2.5 months	Hemispheric	Occipital	Chemotherapy and radiotherapy	Alive	No	7.1 years	Gene fusion	*TPM3-NTRK1*
3	Female	Prenatal (31 weeks of gestation)	Hemispheric	Supratentorial	Chemotherapy	Alive	No	4.8 years	Negative	N/A
4	Female	Prenatal (unavailable data)	Hemispheric	Temporal	Chemotherapy	Alive	No	2.6 years	Negative	N/A

CNS central nervous system, *SNV* single-nucleotide variant, *CNV* copy number variation, *N/A* not applicable

lead to a less aggressive phenotype due to the process of oncogene-induced cell senescence. However, Federal Drug Administration (FDA)-approved therapies specific to this fusion are still lacking and current treatment options for LGGs involve targeting other alterations that dysregulate the *MAPK* pathway (Srinivasa et al. 2020). Consequently, identification of *KIAA1549–BRAF* fusions is essential in the context of clinical trials and treatment management for these patients.

In addition to *KIAA1549–BRAF*, *EWSR1-ATF1* fusion was also identified in one LGG case. Gene fusions involving *EWSR1* gene and members of the CREB family, such as *ATF1* gene, have been reported in a diverse group of tumors. Kao et al. (2017) investigated a cohort of children and young adults, aged 12–23 years old, with myxoid mesenchymal tumors positive for fusions between *EWSR1* and members of the CREB family, including *EWSR1-ATF1* fusion. These data suggest a new brain tumor type entitled intracranial myxoid mesenchymal tumors with *EWSR1* and CREB family gene fusions (*EWSR1-CREB*), and approximately 15 cases have been reported in the literature so far (Kao et al. 2017; De Los et al. 2021; Ballester et al. 2020; Liu et al. 2020). In our study, *EWSR1-ATF1* gene fusion was detected in a PMA sample that affected the optic region from a patient diagnosed with 12 years old. However, this tumor entity has not yet been recognized by the 2021 WHO Classification of Tumors of the CNS.

Recently, it has been proposed six epigenetically different subgroups of childhood HGGs that display alterations in *H3F3A, HIST1H3B, IDH1/2*, and *BRAF* genes (Pollack et al. 2019). H3.3 K27M mutation occurs in 70–80% of childhood HGGs, while H3.1 K27M variant is less frequent (Sturm et al. 2012; Venneti et al. 2013). *IDH* subgroup represents nearly 5% of all pediatric HGGs and tumors with *BRAF* V600E alteration are histologically similar to PXA, accounting for 5–10% of cases. Studies have reported that H3.3 K27M subgroup has a poor long-term survival, while *IDH* and *BRAF* subgroups show a comparatively favorable clinical outcome (Pollack et al. 2019). In our HGG samples, alterations in *H3F3A* gene were the most common events observed, *BRAF* V600E variant was identified in one PXA sample, and no alterations in *IDH1/2* were detected by the panel. Our findings show the presence of recurring molecular alterations, highlighting the genetic and prognostic diversity among these tumors, which consequently may further refine the classification of HGGs in children and adolescents (Pollack et al. 2019).

Genomic profiling analysis of pediatric and adolescent HGGs has shown that K27M and G34R alterations are mutually exclusive and intrinsically associated with the anatomic tumor location, with H3.3 K27M subgroup displaying predilection for the midline brain location, especially for the thalamic region, while H3.3 G34R subgroup

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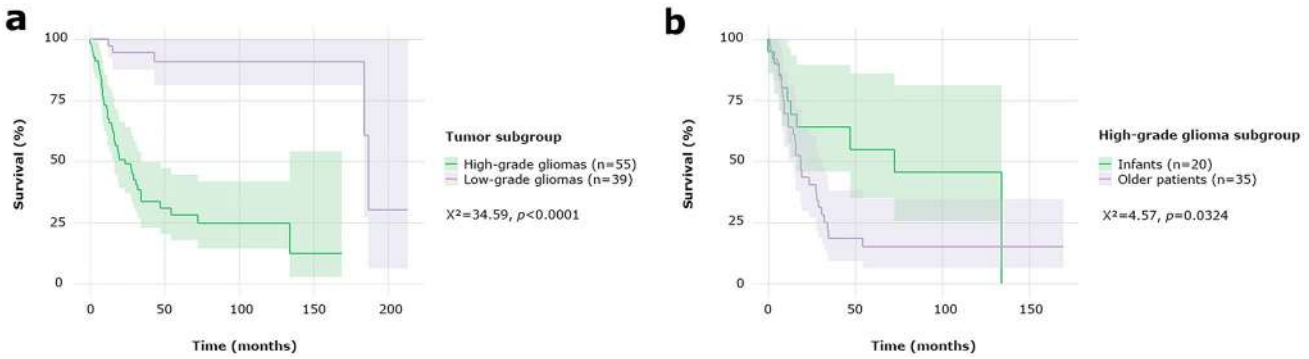

Fig. 4 Overall survival curves comparisons. **a** HGG patients showed a worse 10-year overall survival when compared to LGG patients ($\chi^2 = 34.59$, $p < 0.0001$). **b** HGG patients diagnosed under 5 years old (infant) had a better 10-year overall survival when compared to older patients with HGG ($\chi^2 = 4.57$, $p = 0.0324$)

predominantly affects the cerebral hemisphere (Oliveira et al. 2021; Schwartzentruber et al. 2012). Similarly, among the HGG samples with H3.3 K27M mutation analyzed in our study, 87.5% were midline and the only tumor harboring H3.3 G34R variant was hemispheric. Whereas G34R mutation is highly expressed in the neocortex during the early stages of embryonic development, K27M mutation is associated with the intermediate and late stages of development, mostly of the thalamic region. These evidences indicate that HGGs harboring H3.3 K27M or H3.3 G34R alterations have specific patterns of gene expression during brain development that correlate with the subsequent tumor location. Consequently, K27M and G34R mutations define two clinically and biologically distinct subgroups of childhood HGGs (Sturm et al. 2012; Ebrahimi et al. 2019).

In our analysis, *H3F3A* variants simultaneously overlapped with other specific alterations within the same tumor, including *TP53* (58.8%) and *ATRX* (35.3%) loss-of-function mutations, and amplifications of *MET* (17.6%), *MYC* (17.6%), and *PDGFRA* (23.5%) genes. Approximately 60% of all H3.3 K27M mutations are associated with alterations in *TP53* and 30% in *ATRX* (Deng et al. 2018; Huang et al. 2017; Yuen and Knoepfler 2013). Additionally, it has been shown that tumors harboring H3.3 K27M mutation contain higher numbers of CNVs, with *MYC* and *PDGFRA* gene amplifications being the most commonly observed (Schwartzentruber et al. 2012; Yuen and Knoepfler et al. 2013; Korshunov et al. 2015). A clinically significant finding in our study was the fact that HGG patients with H3.3 K27-altered tumors had a worse 5-year OS in comparison to the wildtype subgroup supporting the evidence that H3.3 K27M mutation is an adverse prognostic factor in pediatric HGGs and these patients may benefit from therapies that target chromatin remodeling agents or post-transcriptional histone modifications.

To date, approximately 120 cGBM cases have been reported and due to its infrequent occurrence, few studies have focused on the molecular and genetic features of this disease (Macy et al. 2012; Espiritu et al. 2020). In the present study, an increase in the number of copies of *ALK* gene and a *PPP1CB-ALK* fusion were identified in one cGBM patient. Interestingly, Zhong et al. (2021) recently reported a case of a female patient with cGBM that also died due to disease progression two days after birth and showed genetic alterations similar to those observed in our patient. They detected an *ALK* amplification and a *PPP1CB-ALK* fusion, which resulted of a local chromothripsis and consisted of exon 1–5 of *PPP1CB* and exon 20–29 of *ALK* (Zhong et al. 2021). Although several studies have shown that *ALK* fusion proteins are present in a diverse group of neoplasms, *PPP1CB-ALK* fusions have been reported in few cases in the literature, mainly with respect to pediatric patients with HGG. (Zhong et al. 2021; Aghajan et al. 2016; Guerreiro Stucklin et al. 2019; Ng et al. 2019). Currently, efforts are underway to develop small molecule tyrosine kinase inhibitors (TKIs) that penetrate the blood–brain barrier, since therapies targeting alterations in *ALK* gene have demonstrated to improve overall outcomes in patients whose CNS tumors arise from these genetic alterations (Holla et al. 2017; Wong 2017). *PPP1CB-ALK* fusions are one of the most common receptor tyrosine kinase fusions in infantile gliomas and recent findings have demonstrated that tumor cells and xenograph tumors positive for these fusions are sensitive to *ALK*-inhibitors (Zhong et al. 2021; Guerreiro Stucklin et al. 2019; Amend et al. 2020). In this context, *PPP1CB-ALK* fusion and *ALK* amplification may represent a set of potential biomarkers for cGBM.

TPM3-NTRK1 fusion, which has already been shown to act as an oncogenic driver in HGG (Hsiao et al. 2019), was identified in one cGBM case in our study. Gliomas can harbor *NTRK* fusions with frequencies of 5–25% and *NTRK* fusions have been reported in up to 40% of non-brainstem HGGs (NBS-HGGs) in patients under 3 year sold (Amatu et al. 2016; Gambella et al. 2020; Clarke et al.

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2020; Wu et al. 2014). Our cGBM patient, whose tumor was located hemispheric and is *TPM3-NTRK1* fusion-positive, was diagnosed with 2.5 months and showed no other significant additional alterations. These results seem to corroborate previous findings that have detected *TPM3-NTRK1* fusions in a particular subset of infant patients with NBS-HGGs (Clarke et al. 2020; Wu et al. 2014). *NTRK* fusion is a pharmacologically targetable genetic alteration and identification of fusion genes responsible for altering the activity of *NTRK* kinase domains has allowed the emergence of specific TKIs for these proteins (Lange and Lo 2018). Thus, multiple TKIs with activity against the tyrosine kinase family are available nowadays or being investigated in clinical trials. The first-generation TKIs, larotrectnib (Vitrakvi®) and entrectinib (Rozlytrek®), represent the two compounds that are furthest in clinical development so far, and were the first FDA-approved TKIs for adult and pediatric solid tumors with *NTRK* fusions (Rolfo 2020; Jiang et al. 2021).

The WHO Classification of Tumors of the CNS, published in 2021, has recognized a novel subgroup of HGG that occurs in newborns and infants with fusions in *ALK, ROS1, NTRK*, or *MET* genes, denoted as infant-type hemispheric glioma (Louis et al. 2021). None of our cGBM cases showed variants in *H3F3A*, *TP53*, and *ATRX* genes, alterations that are frequently seen in older children and adolescents with HGG. Instead, we identified variants in *ALK* and *NTRK* genes, suggesting that cGBM may comprises a specific entity of infant-type hemispheric glioma. Additionally, our survival analysis showed that infant patients with HGG have a more favorable prognosis with improved survival when compared to HGG in older children, which is in concordance with previous reports (Clarke et al. 2020; Gilani et al. 2020). These data indicate that histopathological grading may not truthfully reflect the clinical and biological aspects of these tumors, which highlights the need for an individualized diagnostic and therapeutic care. In such a young group of patients, chemotherapy and radiotherapy are limited approaches due to their significant long-term toxicity and in most cases, surgery and gross total resection represent the main treatment options. Hence, our findings emphasize the demand for age-specific diagnosis and treatment strategies, as identification of molecular alterations may provide potential therapeutic targets for these patients (Guerreiro Stucklin et al. 2019; Espiritu et al. 2020).

In conclusion, molecular profiling by the OCCRA® panel comprehensively addressed the most relevant alterations in gliomas of childhood and adolescence. *KIAA1549–BRAF* fusion was the most significant favorable prognostic factor in LGGs and *H3F3A* c.83A > T variant, commonly detected in HGGs, was associated with a worse OS in these patients. Alterations in *ALK* and *NTRK* genes were identified in two single cases of cGBM, which may provide potential targets for therapy with FDA-approved drugs. Therefore, we were able to detect recurrent somatic mutations in LGGs and HGGs in children and adolescents, as these tumors have specific patterns of molecular alterations, outcomes, and effectiveness to therapies.

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Author contributions Conceptualization and methodology: SRCT, FTG, IDO; Formal analysis and investigation: DCCC, SRCT, FTG, IDO; Writing—original draft preparation: DCCC; Writing—review and editing: SRCT, FTG, NSS, AMC; Funding acquisition: SRCT; Medical support: NSS, AMC, MTSA, PD, SC; Supervision: SRCT.

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

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Research Committee (Committee for Ethics in Research—Federal University of Sao Paulo no. 0915/2019). This article does not contain any studies with animals performed by any of the authors.

Informed consent Samples from each primary tumor were collected after informed consent was signed by patients/guardians according to the Institutional Research Committee (Committee for Ethics in Research—Federal University of Sao Paulo no. 0915/2019). The biological material is acquired via a Biobank of the Pediatric Oncology Institute-GRAACC/UNIFESP (National Commission of Ethics in Research—CONEP B-053).

References

Aghajan Y, Levy ML, Malicki DM, Crawford JR (2016) Novel PPP1CB-ALK fusion protein in a high-grade glioma of infancy. BMJ Case Rep. https://doi.org/10.1136/bcr-2016-217189

Ahmed AA, Vundamati DS, Farooqi MS, Guest E (2018) Precision medicine in pediatric cancer: current applications and future prospects. High Throughput 7(4):39. https://doi.org/10.3390/ht7040039

Amatu A, Sartore-Bianchi A, Siena S (2016) NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. ESMO Open 1(2):e000023. https://doi.org/10.1136/esmoopen-2015-000023

Amend C, Stadler J, Salamat S, Dedekam E, Waanders A, Wadhwani N (2020) Congenital glioblastoma multiforme: a case report of a rare pediatric brain tumor, molecular analysis, and review of the literature. Neuro-Oncol 22(suppl3):iii351. https://doi.org/10.1093/neuonc/noaa222.324

Ballester LY, Meis JM, Lazar AJ et al (2020) Intracranial myxoid mesenchymal tumor with EWSR1-ATF1 fusion. J Neuropathol Exp Neurol 79(3):347–351. https://doi.org/10.1093/jnen/nlz140

Blattner-Johnson M, Jones DTW, Pfaff E (2021) Precision medicine in pediatric solid cancers. Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2021.06.008

Braunstein S, Raleigh D, Bindra R, Mueller S, Haas-Kogan D (2017) Pediatric high-grade glioma: current molecular landscape and therapeutic approaches. J Neurooncol 134(3):541–549. https://doi.org/10.1007/s11060-017-2393-0

Clarke M, Mackay A, Ismer B et al (2020) Infant high-grade gliomas comprise multiple subgroups characterized by novel targetable gene fusions and favorable outcomes. Cancer Discov 10(7):942–963. https://doi.org/10.1158/2159-8290.CD-19-1030

De Los SY, Shin D, Malnik S et al (2021) Intracranial myxoid mesenchymal neoplasms with EWSR1 gene rearrangement: report of 2 midline cases with one demonstrating durable response to MET inhibitor monotherapy. Neurooncol Adv 3(1):vdab016. https://doi.org/10.1093/noajnl/vdab016

Deng H, Zeng J, Zhang T et al (2018) Histone H3.3K27M mobilizes multiple cancer/testis (ct) antigens in pediatric glioma. Mol Cancer Res 16(4):623–633. https://doi.org/10.1158/1541-7786.MCR-17-0460

Drobysheva A, Klesse LJ, Bowers DC et al (2017) Targeted MAPK pathway inhibitors in patients with disseminated pilocytic astrocytomas. J Natl Compr Canc Netw 15(8):978–982. https://doi.org/10.6004/jnccn.2017.0139

Ebrahimi A, Skardelly M, Schuhmann MU et al (2019) High frequency of H3 K27M mutations in adult midline gliomas. J Cancer Res Clin Oncol 145(4):839–850. https://doi.org/10.1007/s00432-018-02836-5

Espiritu AI, Terencio BB, Jamora RDG (2020) Congenital glioblastoma multiforme with long-term childhood survival: a case report and systematic review. World Neurosurg 139:90–96. https://doi.org/10.1016/j.wneu.2020.03.212

Fangusaro J (2012) Pediatric high grade glioma: a review and update on tumor clinical characteristics and biology. Front Oncol 2:105. https://doi.org/10.3389/fonc.2012.00105

Gambella A, Senetta R, Collemi G, Vallero SG, Monticelli M, Cofano F, Zeppa P, Garbossa D, Pellerino A, Rudà R, Soffietti R, Fagioli F, Papotti M, Cassoni P, Bertero L (2020) NTRK fusions in central nervous system tumors: a rare, but worthy target. Int J Mol Sci 21(3):753. https://doi.org/10.3390/ijms21030753

Gilani A, Donson A, Davies KD, Whiteway SL, Lake J, DeSisto J, Hoffman L, Foreman NK, Kleinschmidt-DeMasters BK, Green AL (2020) Targetable molecular alterations in congenital glioblastoma. J Neurooncol 146(2):247–252. https://doi.org/10.1007/s11060-019-03377-8

Guerreiro Stucklin AS et al (2019) Alterations in ALK/ROS1/NTRK/MET drive a group of infantile hemispheric gliomas. Nat Commun 10(1):4343. https://doi.org/10.1038/s41467-019-12187-5

Hargrave D (2009) Paediatric high and low grade glioma: the impact of tumour biology on current and future therapy. Br J Neurosurg 23(4):351–363. https://doi.org/10.1080/02688690903158809

Hiemenz MC, Ostrow DG, Busse TM et al (2018) OncoKids: a comprehensive next-generation sequencing panel for pediatric malignancies. J Mol Diagn 20(6):765–776. https://doi.org/10.1016/j.jmoldx.2018.06.009

Holla VR, Elamin YY, Bailey AM et al (2017) ALK: a tyrosine kinase target for cancer therapy. Cold Spring Harb Mol Case Stud 3(1):a001115. https://doi.org/10.1101/mcs.a001115

Hsiao SJ, Zehir A, Sireci AN, Aisner DL (2019) Detection of tumor NTRK gene fusions to identify patients who may benefit from tyrosine kinase (TRK) inhibitor therapy. J Mol Diagn 21(4):553–571. https://doi.org/10.1016/j.jmoldx.2019.03.008

Huang TY, Piunti A, Lulla RR et al (2017) Detection of Histone H3 mutations in cerebrospinal fluid-derived tumor DNA from children with diffuse midline glioma. Acta Neuropathol Commun 5(1):28. https://doi.org/10.1186/s40478-017-0436-6

Jiang T, Wang G, Liu Y et al (2021) Development of small-molecule tropomyosin receptor kinase (TRK) inhibitors for NTRK fusion cancers. Acta Pharm Sin B 11(2):355–372. https://doi.org/10.1016/j.apsb.2020.05.004

Jones DT, Kocialkowski S, Liu L, Pearson DM, Bäcklund LM, Ichimura K, Collins VP (2008) Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res 68(21):8673–8677. https://doi.org/10.1158/0008-5472.CAN-08-2097

Jones DT, Gronych J, Lichter P, Witt O, Pfister SM (2012) MAPK pathway activation in pilocytic astrocytoma. Cell Mol Life Sci 69(11):1799–1811. https://doi.org/10.1007/s00018-011-0898-9

Kao YC, Sung YS, Zhang L et al (2017) EWSR1 fusions with creb family transcription factors define a novel myxoid mesenchymal tumor with predilection for intracranial location. Am J Surg Pathol 41(4):482–490. https://doi.org/10.1097/PAS.0000000000000788

Kaul A, Chen YH, Emnett RJ, Dahiya S, Gutmann DH (2012) Pediatric glioma-associated KIAA1549:BRAF expression regulates neuroglial cell growth in a cell type-specific and mTOR-dependent manner. Genes Dev 26(23):2561–2566. https://doi.org/10.1101/gad.200907.112

Korshunov A, Ryzhova M, Hovestadt V et al (2015) Integrated analysis of pediatric glioblastoma reveals a subset of biologically favorable tumors with associated molecular prognostic markers. Acta Neuropathol 129(5):669–678. https://doi.org/10.1007/s00401-015-1405-4

Lange AM, Lo HW (2018) Inhibiting TRK proteins in clinical cancer therapy. Cancers (basel) 10(4):105

Liu C, Liu Y, Zhao Y et al (2020) Primary intracranial mesenchymal tumor with ewsr1-crem gene fusion: a case report and literature review. World Neurosurg 142:318–324. https://doi.org/10.1016/j.wneu.2020.07.015

Lorentzian A, Biegel JA, Ostrow DG et al (2018) Tumor variant identification that accounts for the unique molecular landscape of pediatric malignancies. JNCI Cancer Spectr 2(4):pky079. https://doi.org/10.1093/jncics/pky079

Louis DN, Perry A, Wesseling P et al (2021) The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol 23(8):1231–1251. https://doi.org/10.1093/neuonc/noab106

Macy ME, Birks DK, Barton VN, Chan MH, Donson AM, Kleinschmidt-Demasters BK, Bemis LT, Handler MH, Foreman NK (2012) Clinical and molecular characteristics of congenital glioblastoma. Neuro Oncol 14(7):931–941. https://doi.org/10.1093/neuonc/nos125

Ng A, Levy ML, Malicki DM, Crawford JR (2019) Unusual high-grade and low-grade glioma in an infant with PPP1CB-ALK gene

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fusion. BMJ Case Rep 12(2):e228248. https://doi.org/10.1136/bcr-2018-228248

Oliveira VF, De Sousa GR, Dos Santos AC et al (2021) Evaluating H3F3A K27M and G34R/V somatic mutations in a cohort of pediatric brain tumors of different and rare histologies. Childs Nerv Syst 37(2):375–382. https://doi.org/10.1007/s00381-020-04852-8

Pollack IF, Agnihotri S, Broniscer A (2019) Childhood brain tumors: current management, biological insights, and future directions. J Neurosurg Pediatr 23(3):261–273. https://doi.org/10.3171/2018.10.PEDS18377

Rolfo C (2020) NTRK gene fusions: a rough diamond ready to sparkle. Lancet Oncol 21(4):472–474. https://doi.org/10.1016/S1470-2045(20)30026-7

Schwartzentruber J, Korshunov A, Liu XY et al (2012) Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature 482(7384):226–231. https://doi.org/10.1038/nature10833

Severino M, Schwartz ES, Thurnher MM, Rydland J, Nikas I, Rossi A (2010) Congenital tumors of the central nervous system. Neuroradiology 52(6):531–548. https://doi.org/10.1007/s00234-010-0699-0

Solitare GB, Krigman MR (1964) Congenital intracranial neoplasm. A case report and review of the literature. J Neuropathol Exp Neurol 23:280–292

Srinivasa K, Cross KA, Dahiya S (2020) BRAF alteration in central and peripheral nervous system tumors. Front Oncol 10:574974. https://doi.org/10.3389/fonc.2020.574974

Sturm D, Witt H, Hovestadt V et al (2012) Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell 22(4):425–437. https://doi.org/10.1016/j.ccr.2012.08.024

Sturm D, Bender S, Jones DT et al (2014) Paediatric and adult glioblastoma: multiform (epi)genomic culprits emerge. Nat Rev Cancer 14(2):92–107. https://doi.org/10.1038/nrc3655

Terashima K, Ogiwara H (2021) No shinkei geka. Neurol Surg 49(3):640–646. https://doi.org/10.11477/mf.1436204438

Venneti S, Garimella MT, Sullivan LM et al (2013) Evaluation of histone 3 lysine 27 trimethylation (H3K27me3) and enhancer of Zest 2 (EZH2) in pediatric glial and glioneuronal tumors shows decreased H3K27me3 in H3F3A K27M mutant glioblastomas. Brain Pathol 23(5):558–564. https://doi.org/10.1111/bpa.12042

Wen PY, Packer RJ (2021) The 2021 WHO classification of tumors of the central nervous system: clinical implications. Neuro Oncol 23(8):1215–1217. https://doi.org/10.1093/neuonc/noab120

Wong A (2017) The emerging role of targeted therapy and immunotherapy in the management of brain metastases in non-small cell lung cancer. Front Oncol 7:33. https://doi.org/10.3389/fonc.2017.00033

Wu G, Diaz AK, Paugh BS et al (2014) The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. Nat Genet 46(5):444–450. https://doi.org/10.1038/ng.2938

Yuen BT, Knoepfler PS (2013) Histone H3.3 mutations: a variant path to cancer. Cancer Cell 24(5):567–574. https://doi.org/10.1016/j.ccr.2013.09.015

Zhong Y, Lin F, Xu F, Schubert J, Wu J, Wainwright L, Zhao X, Cao K, Fan Z, Chen J, Lang SS, Kennedy BC, Viaene AN, Santi M, Resnick AC, Storm PB, Li MM (2021) Genomic characterization of a PPP1CB-ALK fusion with fusion gene amplification in a congenital glioblastoma. Cancer Genet 252–253:37–42. https://doi.org/10.1016/j.cancergen.2020.12.005

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