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Clinicopathological analysis of BRAF and non-BRAF MAPK pathway-altered gliomas in paediatric and adult patients: a single-institution study of 40 patients

Rola H Ali ,[1,2] Mohamad Almanabri ,[3] Nawal Y Ali ,[4] Ahmad R Alsaber ,[5] Nisreen M Khalifa,[6] Rania Hussein,[7] Mona Alateeqi,[8] Eiman M A Mohammed ,[8] Hiba Jama,[8] Ammar Almarzooq,[8] Noelle Benobaid,[8] Zainab Alqallaf,[8] Amir A Ahmed ,[8] Shakir Bahzad,[8] Maryam Almurshed [2]

[1]Department of Pathology, College of Medicine, Kuwait University, Jabriya, Hawalli, Kuwait
[2]Department of Histopathology, Al Sabah Hospital, Shuwaikh, Al Asimah, Kuwait
[3]Department of Neurosurgery, Ibn Sina Hospital, Shuwaikh, Al Asimah, Kuwait
[4]Department of Radiology, Ibn Sina Hospital, Shuwaikh, Al Asimah, Kuwait
[5]Department of Management, College of Business and Economics, American University of Kuwait, Salmiya, Hawalli, Kuwait
[6]Department of Pediatric Hematology/Oncology, NBK Children's Hospital, Shuwaikh, Al Asimah, Kuwait
[7]Department of Radiation Oncology, Kuwait Cancer Control Center, Shuwaikh, Al Asimah, Kuwait
[8]Molecular Genetics Laboratory, Kuwait Cancer Control Center, Shuwaikh, Al Asimah, Kuwait

Correspondence to
Dr Rola H Ali; rola.ali@ku.edu.kw

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ABSTRACT

Aims Mitogen-activated protein kinase (MAPK) pathway alteration is a major oncogenic driver in paediatric low-grade gliomas (LGG) and some adult gliomas, encompassing BRAF (most common) and non-BRAF alterations. The aim was to determine the frequency, molecular spectrum and clinicopathological features of MAPK-altered gliomas in paediatric and adult patients at our neuropathology site in Kuwait.

Methods We retrospectively searched the data of molecularly sequenced gliomas between 2018 and 2023 for MAPK alterations, revised the pathology in view of the 2021 WHO classification and evaluated the clinicopathological data for possible correlations.

Results Of 272 gliomas, 40 (15%) harboured a MAPK pathway alteration in 19 paediatric (median 9.6 years; 1.2–17.6) and 21 adult patients (median 37 years; 18.9–89.2), comprising 42% and 9% of paediatric and adult cases, respectively. Pilocytic astrocytoma and glioblastoma were the most frequent diagnoses in children (47%) and adults (43%), respectively. BRAF V600E (n=17, 43%) showed a wide distribution across age groups, locations and pathological diagnoses while KIAA1549::BRAF fusion (n=8, 20%) was spatially and histologically restricted to cerebellar paediatric LGGs. Non-V600E variants and BRAF amplifications accompanied other molecular aberrations in high-grade tumours. Non-BRAF MAPK alterations (n=8) included mutations and gene fusions involving FGFR1, NTRK2, NF1, ROS1 and MYB. Fusions included KANK1::NTRK2, GOPC::ROS1 (both infant hemispheric gliomas), FGFR1::TACC1 (diffuse LGG), MYB::QKI (angiocentric glioma) and BCR::NTRK2 (glioblastoma). Paradoxical H3 K27M/MAPK co-mutations were observed in two LGGs.

Conclusion The study provided insights into MAPK-altered gliomas in Kuwait highlighting the differences among paediatric and adult patients and providing a framework for planning therapeutic polices.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Mitogen-activated protein kinase (MAPK) signalling pathway is a major driver of gliomagenesis particularly in children and is an attractive therapeutic target for MAPK pathway inhibitors.
⇒ The prototypic BRAF V600E mutation and KIAA1549::BRAF fusion are the best characterised among the MAPK alterations in gliomas while non-canonical BRAF and non-BRAF alterations are not fully elucidated.
⇒ The molecular landscape and clinicopathological characteristics of MAPK-altered gliomas are unknown in Kuwait.

WHAT THIS STUDY ADDS

⇒ MAPK pathway alterations beyond BRAF V600E and KIAA1549::BRAF encompass a heterogeneous group of neoplasms.
⇒ There are significant differences in the molecular spectrum and clinicopathological characteristics between paediatric and adult MAPK-altered gliomas.
⇒ Adult gliomas harbour a broader range of MAPK alterations associated with increased genomic complexity and a predominance of high-grade histology.
⇒ Non-BRAF MAPK-related driver alterations are relatively rare but are important to identify due to potential treatment implications.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study has improved our understanding of MAPK pathway-altered gliomas in our population, which should help guide future therapeutic strategies.

INTRODUCTION

Mitogen-activated protein kinase (MAPK) cascades are signalling pathways that regulate diverse cellular processes.[1] The RAS/RAF/MEK/ERK cascade, the best characterised of the MAPK pathways, is triggered by ligand-mediated activation of receptor tyrosine kinases (RTKs), followed by sequential activation of downstream kinases. Oncogenic mutations at various levels in the cascade drive human carcinogenesis and serve as potential therapeutic targets. BRAF (v-raf murine viral oncogene homolog B), one of the most frequently mutated

Table 1 Overall characteristics of the cohort

Variable	No (%)
Age at diagnosis	
<18 years	19 (47.5)
	Median 9.6
	Range 1.2–17.6
≥18 years	21 (52.5)
	Median 37
	Range 18.9–89.2
Gender	
Male	25 (62.5)
Female	15 (37.5)
Tumour site	
Cerebral hemispheres	27 (67.5)
Diencephalon	3 (7.5)
Cerebellum	7 (17.5)
Brain stem	3 (7.5)
Integrated diagnosis WHO 2021	
Circumscribed astrocytic glioma	
PA	9 (22.5)
PXA	4 (10)
Paediatric-type diffuse LGG	
PLNTY	3 (7.5)
DLGG, MAPK	4 (10)
Angiocentric glioma	1 (2.5)
Paediatric-type diffuse HGG	
Infant-type hemispheric glioma*	2 (5)
Adult-type diffuse glioma	
GBM	9 (22.5)
ODG	1 (2.5)
Astrocytoma	1 (2.5)
Glioneuronal tumour	
GG	2 (5)
Not elsewhere classified	4 (10)
Histological grade	
Low (1 and 2)	24 (60)
High (3 and 4)	16 (40)
Total	40

*Included one low grade and one high grade.
DLGG, MAPK, diffuse low-grade glioma MAPK pathway-altered; GBM, glioblastoma; GG, ganglioglioma; HGG, high-grade glioma; LGG, low-grade glioma; ODG, oligodendroglioma IDH-mutant 1p/19q-codeleted; PA, pilocytic astrocytoma; PLNTY, polymorphous low-grade neuroepithelial tumour of the young; PXA, pleomorphic xanthoastrocytoma.

genes in cancer, encodes a downstream serine/threonine kinase in the MAPK pathway.[2]

Accurate characterisation of oncogenic BRAF alterations is necessary for optimising treatment regimens and minimising side effects such as paradoxical acceleration of tumour growth.[3–5] Three functional classes of cancer-associated BRAF mutations determine sensitivity to RAF inhibitors.[6–8] Class I mutants, exemplified by the hotspot V600E mutation, are RAS-independent constitutively active kinase monomers conferring sensitivity to first-generation 'monomer' RAF inhibitors (eg, vemurafenib and dabrafenib).[6] In contrast, class II (RAS-independent constitutive dimers) and class III (RAS-dependent hypoactive mutants) are insensitive to standard RAF inhibitors,[6 7] hence therapies must be tailored to the specific mutational context.[3]

BRAF alterations are highly prevalent in paediatric low-grade gliomas (LGGs)[9–12] but are also observed in some LGG and high-grade gliomas (HGGs) in adults.[8 10] The canonical BRAF V600E, with a valine-to-glutamic acid substitution at position 600, is the most frequent single-nucleotide variant (SNV) in BRAF in adult and paediatric gliomas overall,[13] while KIAA1549::BRAF, the result of a tandem duplication at 7q34,[14] is specifically predominant in pilocytic astrocytoma (PA) in children. BRAF alterations, however, encompass a broad range of histopathological entities[15] with variability in molecular spectrum and morphology.[10 13] Clinical outcome is also variable depending on other clinicopathological factors particularly age, tumour location, extent of surgery, histological grade and accompanying molecular aberrations.[16–19]

Non-BRAF MAPK alterations are being increasingly identified through next-generation sequencing. These include mutations or gene fusions in RTKs (eg, FGFR1/2/3,[20 21] NTRK1/2/3,[22 23] ROS1,[24 25] ALK[24]) and/or downstream effectors (eg, NF1,[12] a negative regulator of RAS and the transcription factor MYB).[21] RTK inhibition is a promising goal in RTK-altered gliomas, particularly in paediatric gliomas which are usually driven by single-gene alterations, however, there are still many challenges ahead.[26 27]

In the fifth edition of the WHO classification of Central Nervous System (CNS) tumors (2021),[15] MAPK alterations are incorporated as a general category named 'diffuse LGG, MAPK pathway-altered', encompassing tumour subtypes with an astrocytic or oligodendroglial morphology that require molecular characterisation for precise classification. Additionally, more 'definitional' MAPK alterations are incorporated under specific histopathological entities.[28] And yet, the molecular landscape and clinicopathological features of MAPK-altered gliomas have not been fully investigated. This study, conducted at the main neuropathology site in Kuwait, aimed to explore the molecular range of MAPK alterations in a cohort of 332 gliomas with attention to the differences between paediatric and adult tumours and alterations beyond the canonical BRAF V600E. The purpose was to gain insights into the frequencies and characteristics of such tumours in our region for future planning of treatment strategies.

METHODS

Cohort selection and pathological features

We retrospectively searched the archives of the Department of Pathology at Al Sabah hospital, a major neuropathology site in Kuwait, for molecularly characterised glial tumours with a BRAF or other MAPK-related gene alteration diagnosed between 2018 and 2023. Pathology slides were re-evaluated by a neuropathologist refining the diagnoses in accordance with the 2021 WHO classification of CNS tumours.[15]

Next-generation sequencing data

Available molecular sequencing data were compiled from the pathology records, including SNV, small insertions/deletions (indel), copy number variants (CNV) and structural rearrangements. The sequencing was previously performed on formalin-fixed paraffin-embedded pathology samples using the Oncomine Comprehensive Assay v3 (OCAv3) (Thermo Fisher Scientific), a targeted panel covering 161 cancer-associated genes and currently used in our routine clinical setting.[29] In brief, DNA and RNA were extracted simultaneously using RecoverAll Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, USA). The quantity of the extracted DNA and RNA samples was measured on a Qubit 3.0 Fluorometer and adjusted to 20 ng and

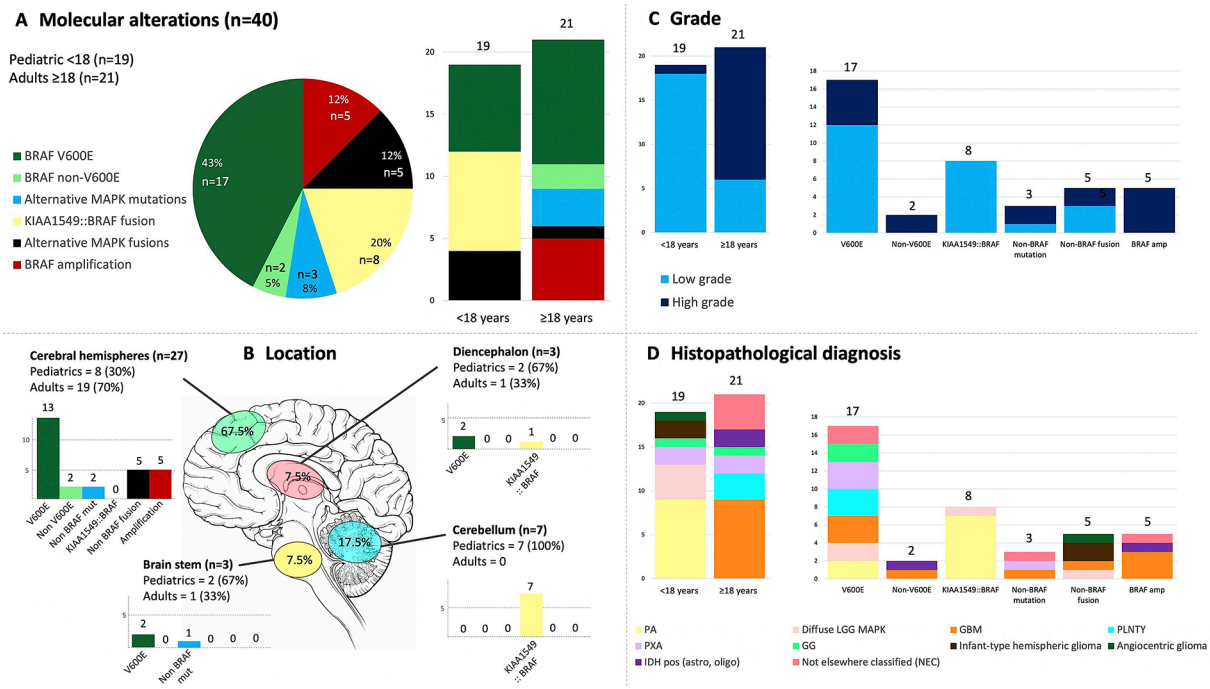

Figure 1 Schematic representation of the clinicopathological findings. (A) Overall frequencies of MAPK gene alterations among paediatric and adult patients. (B) Anatomical locations in relation to molecular alteration type. (C, D) Histological grade and diagnosis in relation to age and molecular alteration type, respectively. Diffuse LGG MAPK, low-grade glioma MAPK pathway-altered; GBM, glioblastoma; GG, ganglioglioma; PA, pilocytic astrocytoma; PLNTY, polymorphous low-grade neuroepithelial tumour of the young; PXA, pleomorphic xanthoastrocytoma.

100 ng, respectively, per sample input. Library preparation was conducted manually following the manufacturer's instructions with the Ion AmpliSeq Library Kit Plus (Thermo Fisher Scientific, USA). The libraries were subsequently sequenced on the Ion Torrent S5 XL platform (Thermo Fisher Scientific, USA), and the resulting data were mapped to human genome assembly 19 (hg19). Sample quality was assessed based on the assay target regions in Ion Server (V.5.18), and data analysis was performed with Ion Reporter Software (V.5.10) (Thermo Fisher Scientific, USA).

Clinical and imaging data

Clinical data collected retrospectively included demographic, neurosurgical and radiological data along with adjuvant treatment and follow-up. Paediatric and adult age groups were defined as patients <18 and ≥18 years at first presentation, respectively. Qualitative MRI characteristics of primary and recurrent tumours were evaluated by a neuroradiologist on T2-weighted, diffusion-weighted imaging, fluid attenuated inversion recovery (FLAIR) mismatch and postcontrast T1-weighted MRI sequences.

Statistical analysis

Descriptive statistics (average, median and range) were performed for continuous variables when appropriate. The Wilcoxon rank sum test, a non-parametric alternative to the t-test, was used for comparing the distributions of continuous data, while Fisher's exact test was used for categorical data in view of the small sample size. The statistical analysis was conducted using JAMOVI V.2.4.8.0. Statistical significance was defined as a $p<0.05$.

RESULTS

Cohort characteristics

Out of 332 glial/glioneuronal tumours identified in the pathology records between 2018 and early 2023, 272 were molecularly characterised by next-generation sequencing, comprised of 17% paediatric and 83% adult gliomas. Forty (15%) cases were found to harbour a MAPK pathway-related alteration, with BRAF constituting the great majority (n=32, 12%). At the initial presentation, there were 21 adults (median age 37 years, range 18.9–89.2), and 19 paediatric patients (median age 9.6 years, range 1.2–17.6) with 5:3 male:female ratio. Most tumours involved the cerebral hemispheres (n=27, 67.5%), followed by cerebellum (n=7, 17.5%), diencephalon (n=3, 7.5%) and brain stem (n=3, 7.5%). The overall characteristics of the cases are summarised in table 1.

Spectrum and characteristics of MAPK pathway alterations

MAPK gene alterations were divided into BRAF (n=32, 80%) and non-BRAF (n=8, 20%) which involved FGFR1, NTRK2, NF1, ROS1 and MYB genes. BRAF V600E mutation was the most common alteration (n=17, 43%), followed by KIAA1549::BRAF fusion (n=8, 20%), BRAF amplifications (n=5, 12%), non-BRAF fusions (n=5, 12%), non-BRAF mutations (n=3, 8%) and non-V600E BRAF variants (n=2, 5%). Figure 1 provides a schematic overview of the distribution of the alterations across age groups, locations, histological categories and grades.

BRAF V600E (class I) and KIAA1549::BRAF (class II) showed statistically significant differences in age, tumour size, location and pathological diagnosis (table 2). KIAA1549::BRAF tumours occurred exclusively in patients <18 (average 6.74 years), were larger in size (average 5.16 cm), midline cerebellar in location and mostly PAs histologically. In contrast, BRAF V600E tumours were more frequent among patients ≥18 (average 25.01 years), were smaller (average 3.29 cm), more likely located in hemispheric regions and encompassed a spectrum of histological subtypes. Two of three extracerebellar PAs harboured BRAF V600E. Histological grade was not significantly different as

Table 2 Contrasting the KIAA1549::BRAF and V600E groups

Variable	Overall N=25	*KIAA1549::BRAF* N=8	*V600E* N=17	P value	q-value*
Age	19.16 (average)	6.74 (average)	25.01 (average)	<0.001†	0.002
Age group				0.008†	0.019
<18	15/25 (60%)	8/8 (100%)	7/17 (41%)		
≥18	10/25 (40%)	0/8 (0%)	10/17 (59%)		
Gender				0.41	0.59
Female	12/25 (48%)	5/8 (62%)	7/17 (41%)		
Male	13/25 (52%)	3/8 (38%)	10/17 (59%)		
Tumour size	3.91 (average)	5.16 (average)	3.29 (average)	0.025†	0.050
Tumour location				<0.001†	0.002
Hemispheric	13/25 (52%)	0/8 (0%)	13/17 (76%)		
Midline	12/25 (48%)	8/8 (100%)	4/17 (24%)		
Path diagnosis of PA				<0.001†	0.002
PA	9/25 (36%)	7/8 (88%)	2/17 (12%)		
Other	16/25 (64%)	1/8 (12%)	15/17 (88%)		
Path diagnosis of GBM				0.53	0.66
GBM	3/25 (12%)	0/8 (0%)	3/17 (18%)		
Other	22/25 (88%)	8/8 (100%)	14/17 (82%)		
Grade				0.14	0.23
Low	20/25 (80%)	8/8 (100%)	12/17 (71%)		
High	5/25 (20%)	0/8 (0%)	5/17 (29%)		

*q-value estimates false discovery rate.
†Indicates statistical significance.
GBM, glioblastoma; PA, pilocytic astrocytoma.

all KIAA1549::BRAF and most V600E tumours were low grade. V600E-mutated HGGs included: epithelioid GBM (n=3), high-grade PXA (n=1) and HGG not elsewhere classified (n=1). Regarding associations with MRI patterns, KIAA1549::BRAF tumours (n=7) showed more prominent cystic changes compared with V600E cases (n=11) (p=0.017), but no statistically significant differences with respect to quality of tumour borders, diffusion restriction, T2/FLAIR mismatch, haemorrhage/calcification or post-contrast enhancement. BRAF V600E and KIAA1549::BRAF were the sole molecular alterations in most cases indicating their driver oncogenic nature (figure 2).

Non-V600E BRAF variants were detected in two HGGs: a class II mutation at p.K601E (GBM grade 4) and a class III mutation at p.K483E (oligodendroglioma grade 3). BRAF amplification was also restricted to HGGs: GBM (n=3), IDH-mutant astrocytoma grade 3 (n=1) and H3 K27M-mutant hemispheric glioma (n=1). Amplification was often accompanied by additional copy number gains particularly of neighbouring genes on chromosome 7q. Clinicopathological details of all BRAF-altered cases are summarised in table 3.

Non-BRAF MAPK pathway-related alterations (n=8) were identified in 4 LGGs and 4 HGGs (median age 22.15, range 1.2–67.6), which included gene fusions and mutations involving FGFR1, NTRK2, NF1, ROS1 and MYB (figure 2). These were mutually exclusive with BRAF alterations. The specific alterations, pathology and clinical details are summarised in table 4. NTRK2 fusions, KANK1::NTRK2 and BCR::NTRK2 occurred in a high-grade infantile and adult glioma, respectively (figure 3), while GOPC::ROS1, MYB::QKI and FGFR1::TACC1 occurred in paediatric LGGs. Non-BRAF alterations were all hemispheric except for one LGG with triple H3 K27M/FGFR1/NF1 mutations (and PA histological phenotype) which involved the brainstem.

Paediatric versus adult MAPK pathway-altered gliomas

The molecular spectrum and clinicopathological characteristics in paediatric patients contrasted with that in adults. Paediatric gliomas mostly harboured BRAF V600E, KIAA1549::BRAF or an alternative non-BRAF fusion, whereas adult gliomas harboured a broader range of alterations including BRAF amplification, non-V600E (Class II/III) variants and non-BRAF mutations (figure 1A). Hemispheric and midline locations showed a significant statistical association with the adult and paediatric age groups, respectively (p=0.0019) (figure 1B). Histological grade was also significantly different with a predominance of LGGs seen in the paediatric group (p<0.001) (figure 1C). Additionally, adult LGGs occurred mainly in patients younger than 35 years. PA was the most common histopathological diagnosis in children (9/19, 47%), while GBM was the most common in adults (9/21, 43%) (figure 1D). Figure 2 is a detailed oncoplot of individual patients contrasting both age groups and showing associated molecular alterations.

The GBM cases in adults (median age 51.4, range 33.2–89.2) showed a spectrum of MAPK alterations including BRAF V600E (n=3), BRAF K601E (n=1), BRAF amplification (n=3), NTRK2 fusion (n=1) and FGFR1 mutation (n=1). All were IDH-wild type and H3F3A-wild type. All three V600E-mutated GBMs showed epithelioid morphology. Almost all GBMs harboured additional genetic alterations but only two had a concurrent EGFR alteration. One GBM had a BCR::NTRK2 fusion as the sole molecular aberration. On the other hand, the PA cases in children (median age 8.9, range 2.3–14.9) showed restriction to KIAA1549::BRAF fusion (n=7) and BRAF V600E (n=2) with no other alterations.

Molecular association with H3 K27M

H3 K27M mutation is a marker of high-grade histology and poor outcome in paediatric midline gliomas. Paradoxically, two

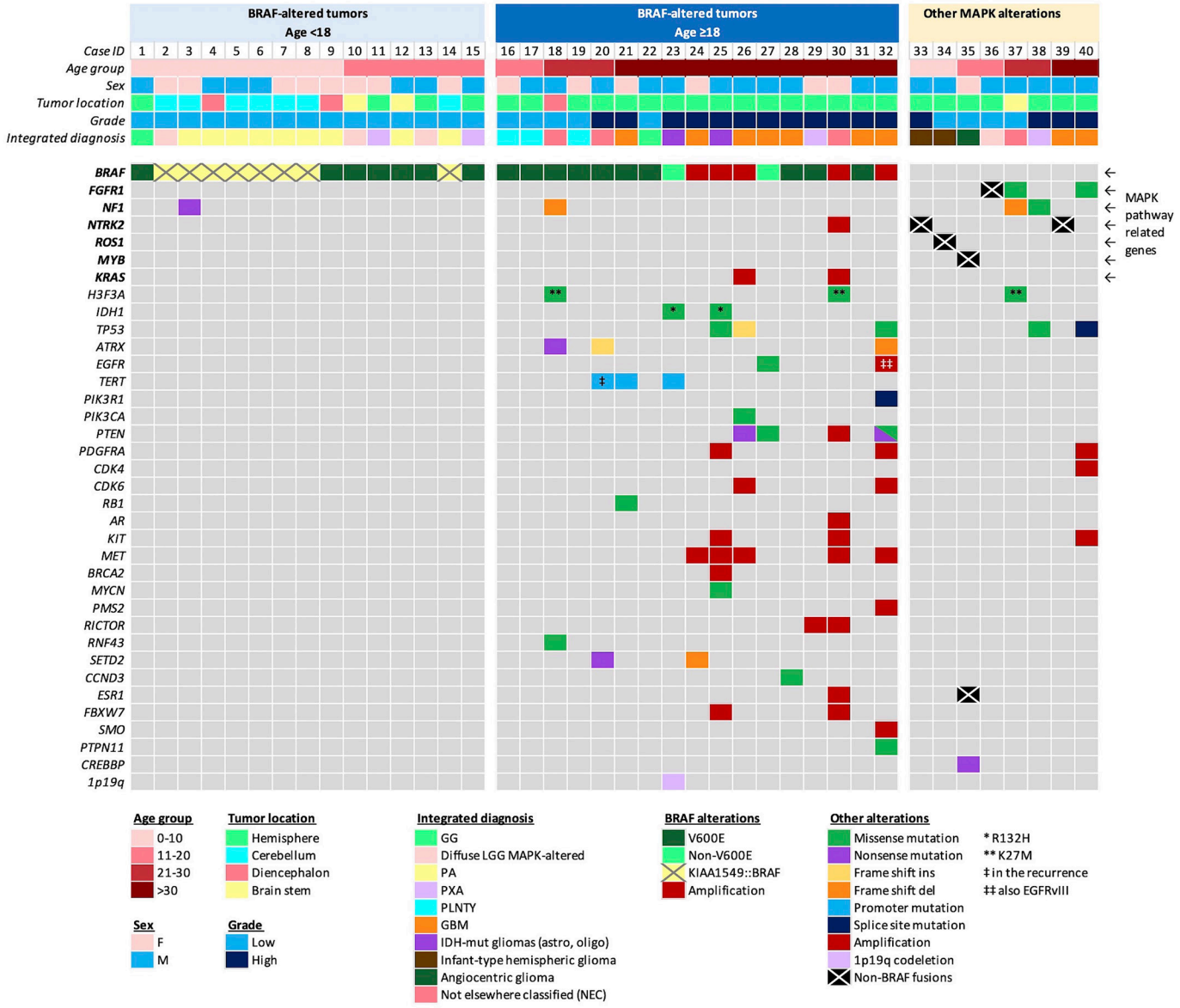

Figure 2 Oncoplot summary, including age, sex, location, grade, pathological diagnosis and molecular alterations. Each column represents a patient while rows represent clinicopathological findings and genes. GBM, glioblastoma; GG, ganglioglioma; Diffuse LGG MAPK, low-grade glioma MAPK pathway-altered; PA, pilocytic astrocytoma; PLNTY, polymorphous low-grade neuroepithelial tumour of the young; PXA, pleomorphic xanthoastrocytoma.

LGG displaying PA histological phenotype showed an H3 K27M mutation in association with a MAPK gene alteration (figure 2). Both occurred in young adults in midline locations and co-harboured more than one mutation: K27M/BRAF/NF1 (case #18) and K27M/FGFR1/NF1 (case #37) (figure 4). On the other hand, a K27M-mutant HGG was identified in an older adult, situated in a hemispheric location, which showed BRAF amplification among multiple other amplifications suggesting that the BRAF aberration in this case is a coincidental passenger event (case #30).

DISCUSSION

MAPK pathway-altered gliomas constitute a heterogeneous group of neoplasms occurring across a wide range of histological subtypes and showing significant differences among paediatric and adult patients with respect to frequency, molecular spectrum and clinicopathological features. The identification of MAPK alterations in glioma has opened the door for novel therapeutic options particularly in paediatric LGGs.

BRAF V600E mutation was the most common alteration overall predominantly in LGGs, in line with published data.[10 13] V600E is known to occur across a variety of glial and glioneuronal tumours such as PXA (40%–90%),[10 30 31] GG (25%–50%),[10 32] PLNTY (30%–40%)[33] and a minority of PAs arising in extracerebellar locations.[10 34] In contrast, the KIAA1549::BRAF fusion was characteristically seen in PA with a strong association with cerebellar location.[35] While paediatric cases showed a high prevalence of BRAF V600E and KIAA1549::BRAF (or alternative non-BRAF fusions) in this study, a wider range of alterations were observed in adults including non-V600E (class II/III) variants, BRAF amplification and non-BRAF mutations. The BRAF p.K601E (class II) mutation identified in this cohort has been previously described in lung cancer and melanoma,[36] where it has shown partial sensitivity to MEK±BRAF inhibitors[37 38] but

Table 3 Clinicopathological data of BRAF-altered cases

ID	Path dx (grade)	BRAF alteration	Site	Adjuvant therapy	Follow-up (months)	Relapse (#)	Relapse interval (months)	Outcome
1	GG (low)	V600E	Hemisphere mesial temporal	None	10.0	None	None	SD
2	DLGG, MAPK (low)	KIAA1549::BRAF	Cerebellum	None	25.4	None	None	SD
3	PA (low)	KIAA1549::BRAF	Cerebellum	None	12.3	None	None	Alive NOS
4	PA (low)	KIAA1549::BRAF	Thalamus	Vincristine, carboplatin	82.1	2	48, 81	PD
5	PA (low)	KIAA1549::BRAF	Cerebellum	None	33.8	1	20	Alive NOS
6	PA (low)	KIAA1549::BRAF	Cerebellum	None	17.8	None	None	SD
7	PA (low)	KIAA1549::BRAF	Consider cerebellum	None	20.1	None	None	Alive NOS
8	PA (low)	KIAA1549::BRAF	Cerebellum	None	8.5	None	None	LFU
9	PA (low)	V600E	Hypothalamus	Vincristine, carboplatin	11.1	1	10.8	SD
10	DLGG, MAPK (low)	V600E	Midbrain	None	20.0	None	None	Alive NOS
11	PXA (low)	V600E	Hemisphere parietal	None	141.6	None	None	Alive NOS
12	PA (low)	V600E	Medulla	RT	46.2	None	None	DOD
13	DLGG, MAPK (low)	V600E	Hemisphere parietal	RT	15.3	3	3.1, 10.2, 14.2	DOD
14	PA (low)	KIAA1549::BRAF	Cerebellum	None	12.5	None	None	Alive NOS
15	PXA (low)	V600E	Hemisphere cortical parietal	None	21.5	None	None	LFU
16	PLNTY (low)	V600E	Hemisphere temporal	None	51.3	None	None	Alive NOS
17	PLNTY (low)	V600E	Hemisphere temporal	None	105.7	1	72	Alive NOS
18	LGG features of PA, H3 K27M, V600E (low)*	V600E	Thalamus	RT, temozolomide	11.2	None	None	LFU
19	PLNTY (low)	V600E	Hemisphere cortical temporal	None	79.0	1	48	NED
20	HGG NOS, IDH-WT, V600E (high)*	V600E	Hemisphere lateral ventricle/temporal	RT, temozolomide	45.6	2	9.8, 43.6	SD
21	GBM, epithelioid (high)	V600E	Hemisphere temporal	RT	16.3	None	None	DOD
22	GG (low)	V600E	Hemisphere temporal	None	71.0	1	54	SD
23	ODG (high)	K483E	Hemisphere parietal	RT, temozolomide	175.5	3	78, 97.4, 158.3	DOD
24	GBM (high)	Amplification	Hemisphere parietal	RT, temozolomide, bevacizumab	45.6	1	12.2	PD
25	Astrocytoma (high)	Amplification	Hemisphere temporal	RT, temozolomide	19.3	None	None	LFU
26	GBM (high)	Amplification	Hemisphere frontal	RT, temozolomide	22.3	None	None	LFU
27	GBM (high)	K601E	Hemisphere temporal	RT, temozolomide, Bevacizumab	23.3	1	16.2	PD
28	GBM, epithelioid (high)	V600E	Hemisphere temporal	RT, temozolomide, bevacizumab	31.4	1	19.2	DOD
29	PXA (high)	V600E	Hemisphere temporal	RT, temozolomide	31.5	1	31.5	PD
30	HGG, hemispheric, H3 K27M, BRAF-amp (high)*	Amplification	Hemisphere mesial temporal	RT, temozolomide, bevacizumab	37.5	1	18.2	LFU
31	GBM, epithelioid (high)	V600E	Hemisphere parietal	RT, temozolomide	38.3	1	28.4	LFU
32	GBM (high)	Amplification	Hemisphere frontal/ parietal/ temporal	None	1.0	None	None	LFU

*Not defined in the 2021 WHO book.
DOD, died of disease; GBM, glioblastoma; GG, ganglioglioma; HGG, high-grade glioma; LFU, lost to follow-up; LGG, low-grade glioma; DLGG, MAPK, diffuse low-grade glioma MAPK pathway-altered; NED, no evidence of disease; Alive NOS, alive not otherwise specified; ODG, oligodendroglioma IDH-mutant 1 p/19q-codeleted; PA, pilocytic astrocytoma; PD, progressive disease; PLNTY, polymorphous low-grade neuroepithelial tumour of the young; PXA, pleomorphic xanthoastrocytoma; RT, radiation therapy; SD, stable disease.

less so in glioma.[13] The other mutation at p.K483E (class III) is less defined and has been sporadically reported in melanoma and rarely GBM.[36 39] Non-V600E BRAF variants and BRAF amplifications accompanied other molecular aberrations in high-grade tumours questioning their role as oncogenic drivers.

Non-BRAF MAPK pathway alterations in this cohort involved the FGFR1, NF1, NTRK2, ROS1 and MYB genes. FGFR1, encoding an RTK, is the second most altered gene in paediatric LGG via mutations/duplications in the tyrosine kinase domain or FGFR1-TACC1 fusions.[40] FGFR1-altered gliomas seem to behave in accordance with their histological grade[41] and may potentially benefit from selective FGFR-selective kinase inhibitors, for example, erdafitinib.[42] In our study, FGFR1 was involved in one FGFR1::TACC1 fusion and two

Table 4 Clinicopathological data of non-BRAF MAPK alterations

ID	Path Dx (grade)	MAPK gene (alteration)	Site	Adjuvant therapy	Follow-up (months)	Relapse (#)	Relapse interval (months)	Outcome
33	Infant-type hemispheric glioma (high)	NTRK2 (KANK1::NTRK2)	Hemisphere temporal	Combination chemo	22.3	1	21.3	PD
34	Infant-type hemispheric glioma (low)	ROS1 (GOPC::ROS1)	Hemisphere temporal	None	21.3	None	None	Alive NOS
35	Angiocentric glioma (low)	MYB (MYB::QKI)	Hemisphere frontal	NA	NA	NA	NA	NA
36	DLGG, MAPK (low)	FGFR1 (FGFR1::TACC1)	Hemisphere parietal	None	5.0	None	None	NED
37	LGG features of PA, H3 K27M (low)*	FGFR1 (N577K) NF1 (Y1680fs)	Brain stem-4th ventricle	RT, bevacizumab	32.4	2	12, 22.3	PD
38	PXA (high)	NF1 (G629R)	Hemisphere frontal	RT, temozolamide	6.1	1	5.1	LFU
39	GBM (high)	NTRK2 (BCR::NTRK2)	Hemisphere temporal	RT, temozolamide	53.0	1	48.8	LFU
40	GBM (high)	FGFR1 (D162A)	Hemisphere parietal	RT, temozolamide	11.1	None	None	LFU

*Not defined in the 2021 WHO book.
Alive NOS, alive not otherwise specified; DLGG, MAPK, diffuse low-grade glioma MAPK pathway-altered; GBM, glioblastoma; LFU, lost to follow-up; NA, not available; NED, no evidence of disease; PD, progressive disease; PXA, pleomorphic xanthoastrocytoma.

mutations. Interestingly, one FGFR1-mutant LGG (p.N577K) co-harboured H3 K27M and NF1 mutations (discussed below). This propensity for additional alterations in FGFR1-mutant tumours was previously observed, either as FGFR1 'dual hits' or additional non-FGFR1 mutations, suggesting a cooperative role in tumourigenesis.[21 40 41] Similarly, NF1 mutations were mostly seen in the company of other known driver alterations, except for one high-grade PXA in a young adult which raised suspicion for neurofibromatosis type 1 (even though PXA is not considered a classic NF1-associated tumour). Of note, no

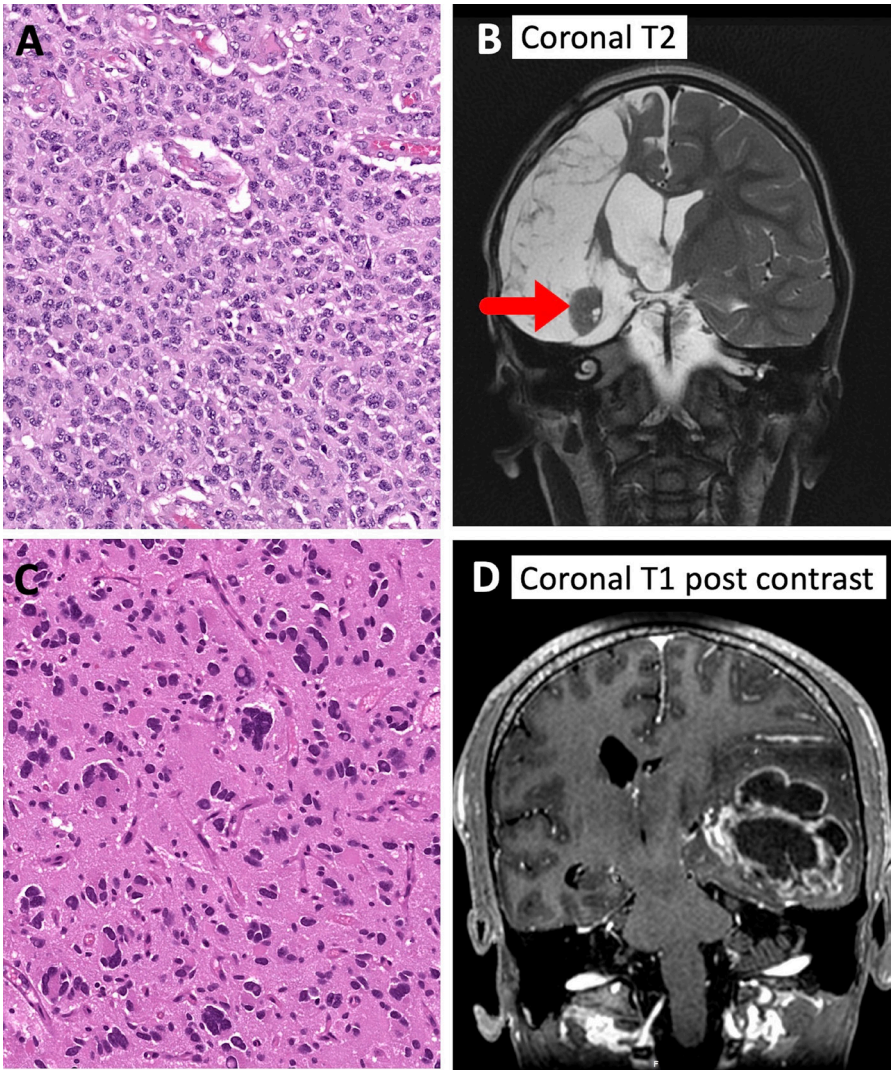

Figure 3 Histopathological and MRI findings in high-grade NTRK2-rearranged gliomas. (A, B) Infant-type hemispheric glioma harbouring KANK1::NTRK2 with postoperative MRI showing a residual nodule (arrow) that has progressed on completion of adjuvant therapy despite the surrounding territorial infarction (case #33). (C, D) Hemispheric glioblastoma in an adult harbouring BCR::NTRK2 with peripheral enhancement on MRI (case #39).

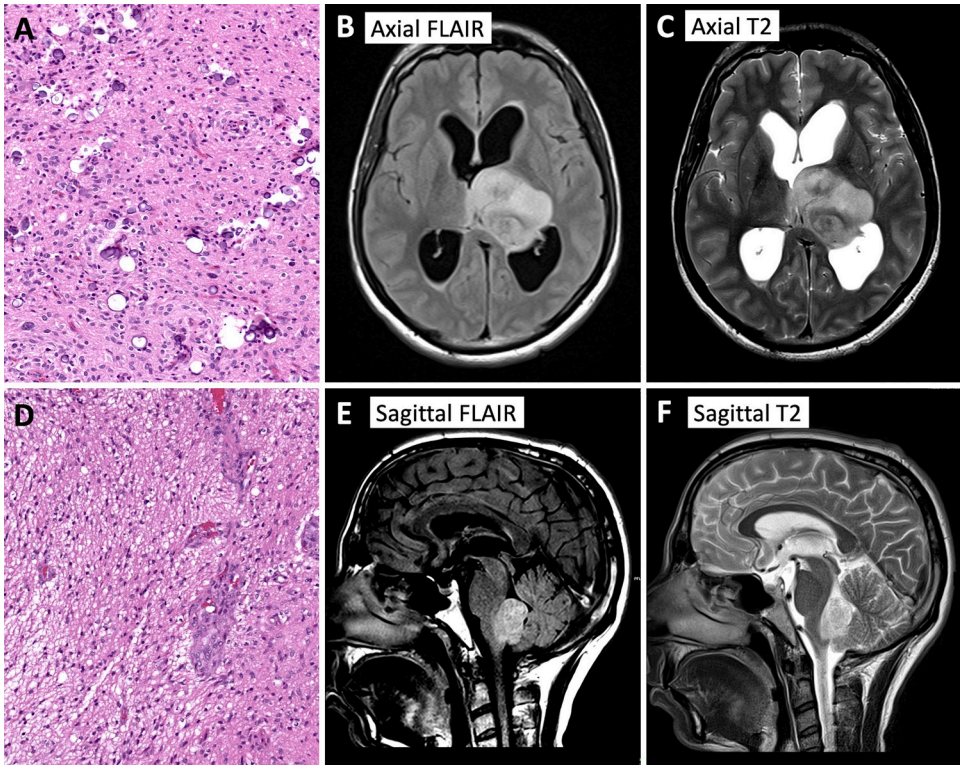

Figure 4 Histopathological and MRI findings in K27M-mutant/MAPK-altered midline gliomas with low-grade pilocytic astrocytoma phenotype. (A–C) A 4.5×4.3 cm tumour involving the left thalamus harbouring K27M/V600E/NF1 mutations (case #18). (D–F) A 2.9×1.9 cm tumour within the fourth ventricle/brain stem harbouring K27M/FGFR1/NF1 mutations (case #37).

NF1-associated optic pathway gliomas were included in this study.

KANK1::NTRK2 and GOPC::ROS1 fusions were identified in two infantile hemispheric gliomas with high and low-grade histology, respectively. Infant gliomas are enriched with RTK fusions, commonly involving ALK, ROS1, NTRK or MET, which seem to confer a relatively better outcome compared with fusion-negative cases.[24 43] NTRK fusions have been previously recognised in a wide age range, while ROS1 were mostly reported in infants.[22 23 25] In a recent multi-institutional study, however, ROS1 fusions in glioma were identified beyond the infant age range, with GOPC being the most frequent partner (77%) across all age groups, although (unlike our case) the infantile tumours were uniformly high grade.[44] The identification of pathognomonic potentially targetable fusions in infantile cases is particularly helpful in view of the diagnostic and therapeutic challenges and paradoxical survival profiles in this age group.[24] Responses to specific TRK inhibitors (eg, crizotinib) have been reported but further validation is needed.[43 45] In our case, the KANK1::NTRK2-fused tumour (high-grade histology) has shown recent disease progression on completion of standard chemotherapy cycles, while the GOPC::ROS1 tumour (low-grade histology) was alive with unknown status.

Interestingly, two midline LGGs with PA histology co-harboured a combination of H3 K27M and MAPK mutations (K27M/V600E/NF1 and K27M/FGFR1/NF1, respectively). Conventional H3 K27-altered glioma, with histone H3 mutation at p.K27M, is a paediatric-type infiltrative HGG localised within the midline and associated with poor outcome.[15] H3 K27M has occasionally been reported in paediatric LGGs including PA[46 47] and ganglioglioma,[48 49] and in association with BRAF V600E,[48–50] and FGFR1 mutations,[41] or as a triple K27M/FGFR1/NF1 combination.[40] The K27M/MAPK co-occurrence suggests a genetic overlap between LGG and HGG with K27M being the dominant driver.[41 48 51] In our case, one patient developed disease progression in 32.4 months while the other was lost to follow-up. Such histological/molecular discrepancy underscores the importance of routine molecular profiling in detecting unexpected patterns and the use of a layered integrated pathology report.[28] A risk-based stratification system combining pathological, molecular and clinical information has been proposed as a more accurate approach for prognostication and management for paediatric-type LGGs.[33 52 53]

The GBM cases in this cohort (all hemispheric and IDH-wild type/H3F3A-wild type) showed a spectrum of MAPK alterations including BRAF V600E, BRAF K601E, BRAF amplification, NTRK2 fusion and FGFR1 mutation. BRAF-mutant GBMs generally lacked concurrent EGFR alterations (which are seen in about 40% of GBMs) suggesting that BRAF-mutant GBMs represent a biologically distinct subset.[39] The prognostic value of BRAF V600E mutation in GBM is unresolved and data regarding BRAF inhibition in this subset of tumours is still insufficient to draw definitive conclusions.[54] Resistance to BRAF inhibitors in GBM is multifactorial and may be due to the presence of class III mutations, reactivation of the MAPK pathway through RAF isoform switching, activation of RTKs or the PI3K pathway among other poorly understood mechanisms.[54] Additionally, intrinsic resistance to targeted therapy may be caused by pre-existing concomitant genetic alterations and physical factors related to blood–brain barrier and tumour microenvironment.[55] Another subset of GBMs is enriched with potentially targetable RTK fusions involving EGFR, NTRK, FGFR and MET with a variety of partners,[23 56–58] yet the effectiveness of tyrosine kinase inhibitors is similarly questionable.[59] In this study a

BCR::NTRK2 fusion was detected in a GBM as the sole molecular event.

There are several limitations in this study. A Cox proportional hazards model did not reach a level of significance to assess survival due to the small sample size in the face of heterogeneous MAPK alterations. Despite this, the study has shed some light on the clinicopathological characteristics of MAPK-altered gliomas in our population, highlighting the differences between paediatric and adult tumours and the importance of an integrative molecular/pathological/clinical approach for patient care. Understanding MAPK alterations is essential for future planning in view of the expanding list of therapeutic options. Long-term follow-up is needed for further understanding of these tumours.

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X Rola H Ali @DrRolaAli, Mohamad Almanabri @manabry44, Nawal Y Ali @Nawal_Akbar, Ahmad R Alsaber @a_alsaber and Amir A Ahmed @DrAmirAAhmed9

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Contributors RHA: conceptualisation, data collection and analysis, manuscript writing and editing, figure preparation and overall content; MAlmanabri, NMK and RH: review of patient charts and data collection; NYA: radiological correlation and figures; ARA: statistical methods and analysis; MAlateeqi, EMAM and HJ: lab investigation and technical assistance; AAA and SB: molecular data analysis and interpretation; MAlmurshed: review of histopathology, figures and overall supervision. All authors critically reviewed and approved the final version of the manuscript.

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ORCID iDs
Rola H Ali http://orcid.org/0000-0001-5747-8900
Mohamad Almanabri http://orcid.org/0009-0008-9120-2658
Nawal Y Ali http://orcid.org/0009-0000-5156-8892
Ahmad R Alsaber http://orcid.org/0000-0001-9478-0404
Eiman M A Mohammed http://orcid.org/0000-0002-8470-7833
Amir A Ahmed http://orcid.org/0000-0001-9276-7230
Maryam Almurshed http://orcid.org/0000-0001-8824-5640

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