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ORIGINAL PAPER

Analysis of *BRAF* V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma

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Abstract Missense mutations of the V600E type constitute the vast majority of tumor-associated somatic alterations in the v-RAF murine sarcoma viral oncogene homolog B1 (*BRAF*) gene. Initially described in melanoma, colon and papillary thyroid carcinoma, these alterations have also been observed in primary nervous system tumors albeit at a low frequency. We analyzed exon 15 of *BRAF* spanning the V600 locus by direct sequencing in 1,320 adult and pediatric tumors of the nervous system including various types of glial, embryonal, neuronal and glioneuronal, meningeal, adenohypophyseal/sellar, and peripheral nervous system tumors. A total of 96 *BRAF* mutations were detected; 93 of the V600E type and 3 cases with a three base pair insertion between codons 599 and 600. The highest frequencies of $BRAF^{V600E}$ mutations were found in WHO grade II pleomorphic xanthoastrocytomas (42/64; 66%) and pleomorphic xanthoastrocytomas with anaplasia (15/23; 65%), as well as WHO grade I gangliogliomas (14/77; 18%), WHO grade III anaplastic gangliogliomas (3/6) and pilocytic astrocytomas (9/97; 9%). In pilocytic astrocytomas $BRAF^{V600E}$ mutation was strongly associated with extra-cerebellar location ($p = 0.009$) and was most frequent in diencephalic tumors (4/12; 33%). Glioblastomas and other gliomas were characterized by a low frequency or

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absence of mutations. No mutations were detected in non-glial tumors, including embryonal tumors, meningiomas, nerve sheath tumors and pituitary adenomas. The high mutation frequencies in pleomorphic xanthoastrocytomas, gangliogliomas and extra-cerebellar pilocytic astrocytomas implicate *BRAF*^{V600E} mutation as a valuable diagnostic marker for these rare tumor entities. Future clinical trials should address whether *BRAF*^{V600E} mutant brain tumor patients will benefit from *BRAF*^{V600E}-directed targeted therapies.

Keywords *BRAF* · V600E mutation · Brain tumor · Pleomorphic xanthoastrocytoma · Ganglioglioma

Introduction

BRAF mutations have been detected in a high proportion of melanoma and papillary thyroid carcinoma and are also frequent in various other cancers, in particular colorectal carcinoma, carcinoma of the biliary tract and ovarian cancer [5, 27, 36]. A comprehensive overview of *BRAF* mutations can be found in the Catalogue of Somatic Mutations in Cancer (http://www.sanger.ac.uk/cosmic) [11]. The vast majority of mutations affects a mutational hot spot at amino acid position 600 and is characterized by the exchange of valine by glutamate (referred to as *BRAF*^{V600E}). Earlier studies of human brain tumors revealed absence of *BRAF*^{V600E} or only single instances of *BRAF*^{V600E} mutations in glioblastoma (GBM) [2, 8, 14, 18, 21] and in astrocytic, oligodendroglial and ependymal tumors of WHO grades II and III [8, 18]. Recent findings, however, suggest that certain types of mostly low grade and pediatric brain tumors may have higher rates of *BRAF* alterations. In particular, pilocytic astrocytomas often carry *BRAF* aberrations, though more commonly in the form of oncogenic fusion genes like *KIAA1549:BRAF* rather than *BRAF*^{V600E} missense mutations [19, 23, 32]. Recently, *BRAF*^{V600E} mutations have been detected in small series of pediatric gangliogliomas, pleomorphic xanthoastrocytomas, desmoplastic infantile gangliogliomas and atypical teratoid/rhabdoid tumors as well as pediatric GBM, anaplastic astrocytomas and diffuse astrocytomas [6, 12, 17, 34].

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BRAF is a member of the RAS/RAF/MEK/ERK kinase pathway. The *BRAF*^{V600E} mutation is thought to mimic phosphorylation of the activating amino acids T599 and S602, thereby leading to constitutive activation of the protein [5]. Such activation affects cell proliferation, differentiation and survival. Several inhibitors targeting the RAS/RAF/MEK/ERK signaling cascade are currently in clinical trials for various malignancies [29]. Furthermore, several small molecules specifically inhibiting the function of *BRAF*^{V600E} have been developed and are currently under investigation. The small molecule inhibitor of *BRAF*^{V600E} PLX4032 (RG7204) has been shown to prolong survival of mice bearing *BRAF*^{V600E} melanoma xenografts [39] and currently is in phase III clinical testing. Similarly, the small molecule inhibitor GDC-0879 has been demonstrated to inhibit tumor growth of melanoma xenografts carrying *BRAF*^{V600E} [15]. Thus, it is of major clinical interest to determine the frequency and distribution of *BRAF*^{V600E} mutations across various types of central and peripheral nervous system tumors to identify those tumor entities that might potentially benefit from *BRAF*^{V600E} targeted therapy. We therefore analyzed a comprehensive series of 1,320 primary pediatric and adult nervous system tumors for the presence of the *BRAF*^{V600E} mutation.

Materials and methods

Tumor specimens

DNA samples from archival human nervous system tumor tissue specimens diagnosed at the Departments of Pathology/Neuropathology at the Universities of Heidelberg, Muenster, Magdeburg, Duesseldorf, the Burdenko Neurosurgical Institute in Moscow, the Radboud University Nijmegen Medical Centre, Nijmegen, and Massachusetts General Hospital, Boston, were analyzed. All tumors were diagnosed according to the revised WHO 2000 and 2007 classifications [26]. In total, 1,384 DNA samples were investigated. Of these, *BRAF* mutation analysis was successful in 1,320 samples (95%); the remaining 64 DNA samples were either not amplifiable by PCR or did not yield evaluable sequences. All samples were analyzed in an anonymous manner as approved by the local ethics committees at the participating institutions.

The series included 97 pilocytic astrocytomas WHO grade I (PA I), 64 pleomorphic xanthoastrocytomas WHO grade II (PXA II), 23 pleomorphic xanthoastrocytomas with anaplasia (aPXA), 57 diffuse astrocytomas WHO grade II, 58 anaplastic astrocytomas WHO grade III (A III), 115 primary glioblastomas WHO grade IV (GBM), 18 secondary glioblastomas WHO grade IV (secGBM, based on clinical information of previously resected lower grade

lesion), 15 giant cell glioblastomas WHO grade IV (gcGBM), 16 gliosarcomas WHO grade IV (GS), 64 oligodendrogliomas WHO grade II (O II), 70 anaplastic oligodendrogliomas WHO grade III, 41 oligoastrocytomas WHO grade II, 51 anaplastic oligoastrocytomas WHO grade III, 5 gliomatosis cerebri, 3 subependymal giant cell astrocytomas WHO grade I (SEGA), 94 ependymomas WHO grade II, 52 anaplastic ependymomas WHO grade III, 4 myxopapillary ependymomas WHO grade I, 2 subependymomas WHO grade I, 141 medulloblastomas WHO grade IV, 29 supratentorial primitive neuroectodermal tumors WHO grade IV, 14 atypical teratoid/rhabdoid tumors WHO grade IV (AT/RT), 77 gangliogliomas WHO grade I (GG I), 6 anaplastic gangliogliomas WHO grade III (GG III), 8 gangliocytomas, 9 central neurocytomas WHO grade II, 4 desmoplastic infantile gangliogliomas or astrocytomas WHO grade I, 4 dysembryoplastic neuroepithelial tumors WHO grade I, 14 schwannomas WHO grade I, 42 meningiomas WHO grade I including the meningothelial and transitional variants, 16 atypical meningiomas WHO grade II, 11 anaplastic meningiomas WHO grade III, 2 hemangiopericytomas WHO grade II, 18 malignant peripheral nerve sheath tumors, 9 neurofibromas, 5 craniopharyngiomas, 4 capillary hemangioblastomas WHO grade I and 58 pituitary adenomas.

The *BRAF* mutation status of 64 PA I has been reported in a previous study [7]. Six of the PXA II samples in this study are also included in a separate study by D. Dias-Santagata et al. (manuscript in preparation). Pediatric tumors were defined as lesions operated in patients younger than 18 years. Diagnoses of the 1,320 tumors are summarized in Table 1. Available data on tumor location of PA I, PXA (both PXA II and aPXA) and GG (both GG I and GG III) is summarized in Table 2.

In 16 tumors (6 GG I, 1 GG III, 3 PXA II, 1 aPXA, 1 O II, 1 PA I, 1 secGBM, 1 gcGBM and 1 GS) with $BRAF^{V600E}$ mutation, peripheral blood samples were available for the investigation of constitutive (germline) or somatic (tumor-associated) origin of the *BRAF* alterations.

DNA from the melanoma cell line A375 with a previously described $BRAF^{V600E}$ mutation served as positive control [39]; HEK293T cells served as wild-type control.

PCR amplification and direct sequencing

Older publications often referred to codon 600 mutations as codon 599 mutations. In 2003, the *BRAF* sequence was updated with the insertion of 3 bp in the coding sequence resulting in a new number of the hot-spot codon (600 instead of 599). In the following, we refer to RefSeq DNA: NM_004333. A fragment of 173 bp length including codon 600 of *BRAF* was amplified using 60 ng each of the sense primer BRAFf TGCTTGCTCTGATAGGAAAATG and the antisense primer BRAFr CCACAAAATGGATCC AGACA. PCR using standard buffer conditions, 100 ng of DNA and GoTaq DNA Polymerase (Promega, Madison, USA) employed 35 cycles with denaturing at 95°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 40 s in a total volume of 25 µl. Two microliters of the PCR amplification product were submitted to the sequencing reaction using the BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, USA). 25 cycles were performed employing 12 ng of the sense primer BRAFf, with denaturing at 95°C for 30 s, annealing at 56°C for 15 s and extension at 60°C for 240 s. For selected cases, a second round of sequencing analysis was performed using the antisense primer BRAFr and the sequencing reaction conditions as described above. Sequences were determined using a semiautomated sequencer (ABI 3100 Genetic Analyzer, Applied Biosystems, Foster City) and the Sequence Pilot version 3.1 software (JSI-Medisys, Kippenheim, Germany). For analysis confirmation, 30 *BRAF*-mutated and 30 wild-type cases were re-analyzed with a second set of primers (sense primer BRAFf_confirm TCATAATGCTTGCTCTGATAGGA and antisense primer BRAFr_confirm GGCCAAAAATTTAATCAGTG GA) generating a 224-bp fragment at the same PCR conditions. All cases demonstrated the same results as in first analysis.

Statistical analysis

Student's *t* test was used to examine the relation of absence or presence of $BRAF^{V600E}$ mutation with age, two-tailed Fisher's exact test was used to examine the relation of $BRAF^{V600E}$ mutation and gender for distinct tumor groups. Two-tailed Fisher's exact test was used to examine associations of tumor location and *BRAF* mutation status for cerebellar versus extra-cerebellar PA I and for temporal versus non-temporal supratentorial GG.

Results

A total of 93 $BRAF^{V600E}$ mutations were detected among the 1,320 tumors with evaluable sequencing results. All mutations were heterozygous with one remaining wild-type allele (Fig. 1). In each mutant case, an exchange of T to A at c.1799 was observed (c.1799T>A). Matched constitutive (leukocyte) DNA was available from 16 patients with $BRAF^{V600E}$ mutant tumors. None of the 16 constitutive DNAs carried a $BRAF^{V600E}$, thus indicating an acquired (somatic) origin of the mutation in the corresponding tumors. In addition to the 93 $BRAF^{V600E}$ mutations, one PXA II was found that carried a three base pair insertion (c.1797_1798insACA) between codon 599 and 600 coding

Table 1 Overview of *BRAF*^{V600E} mutations detected in 1,320 central and peripheral nervous system tumors according to tumor type

Tumor entity/variant	*N* (ad; ped)	*N* V600E (ad; ped)	% V600E (ad; ped)
Glial			
Pilocytic astrocytoma	97 (22; 75)	9 (2; 7)	9% (9%; 9%)
Diffuse astrocytoma	57 (53; 4)	0	0% (0%; 0/4)
Anaplastic astrocytoma	58 (52; 6)	2 (0; 2)	3% (0%; 2/6)
Oligodendroglioma	64 (62; 2)	1 (1; 0)	2% (2%; 0/2)
Anaplastic oligodendroglioma	70 (70; 0)	0	0%
Oligoastrocytoma	41 (41; 0)	0	0%
Anaplastic oligoastrocytoma	51 (51; 0)	0	0%
Primary glioblastoma	115 (79; 36)	2 (0; 2)	2% (0%; 6%)
Secondary glioblastoma	18 (18; 0)	1	6%
Giant cell glioblastoma	15 (15; 0)	1	7%
Gliosarcoma	16 (16; 0)	1	6%
Gliomatosis cerebri	5 (5; 0)	1	1/5
Myxopapillary ependymoma	4 (3; 1)	0	0/4 (0/3; 0/1)
Ependymoma	94 (16; 78)	0	0%
Anaplastic ependymoma	52 (5; 47)	0	0%
Subependymoma	2 (2; 0)	0	0/2
Pleomorphic xanthoastrocytoma	64 (38; 26)	42 (24; 18)	66% (63%; 69%)
Pleomorphic xanthoastrocytoma with anaplasia	23 (13; 10)	15 (5; 10)	65% (38%; 100%)
Subependymal giant cell astrocytoma	3 (3; 0)	1	1/3
Embryonal, neuronal, glioneuronal			
Medulloblastoma	141 (7; 134)	0	0%
CNS primitive neuroectodermal tumor	29 (12; 17)	0	0%
Atypical teratoid/rhabdoid tumor	14 (0; 14)	0	0%
Ganglioglioma	77 (53; 24)	14 (11; 3)	18% (21%; 13%)
Anaplastic ganglioglioma	6 (5; 1)	3 (2/1)	3/6 (2/5; 1/1)
Gangliocytoma	8 (8; 0)	0	0/8
Central neurocytoma	9 (8; 1)	0	0/9
Desmoplastic infantile astrocytoma/ganglioglioma	4 (0; 4)	0	0/4
Dysembryoplastic neuroepithelial tumor	4 (2; 2)	0	0/4
Meningeal and PNS tumors			
Meningioma	42 (42; 0)	0	0%
Atypical meningioma	16 (16; 0)	0	0%
Anaplastic meningioma	11 (11; 0)	0	0%
Hemangiopericytoma	2 (2; 0)	0	0/2
Schwannoma	14 (14; 0)	0	0%
Neurofibroma	9 (8; 1)	0	0/9
Malignant peripheral nerve sheath tumor	18 (16; 2)	0	0%
Others			
Craniopharyngioma	5 (4; 1)	0	0/5
Capillary hemangioblastoma	4 (4; 0)	0	0/4
Pituitary adenoma	58 (57; 1)	0	0%

N number, *ad* adult, *ped* pediatric. Pediatric cases were defined as patients operated before the age of 18 years. Percentages are given for entities with 10 or more investigated tumors. *CNS* central nervous system, *PNS* peripheral nervous system

for threonine (p.T599_V600insT). Two PA I cases also harbored a three base pair insertion coding for threonine between codon 599 and 600, both of which have previously been described in detail elsewhere [7]. The frequencies of *BRAF*^{V600E} mutations in different brain tumor entities are listed in Table 1. Notably, frequent *BRAF*^{V600E} mutations

Table 2 $BRAF^{V600E}$ mutation according to tumor location

Tumor entity (*N*)	Location	*N* ($BRAF^{V600E}$; %)
PA I (94)	Cerebral hemisphere	16 (2; 13%)[a]
	Non-temporal	2 (0)
	Temporal	2 (0)
	Cerebellar	53 (1; 2%)[a]
	Brain stem	10 (1; 10%)
	Diencephalic	12 (4; 33%)
	Optic tract	2 (0)
	Spinal	1 (1)
GG (69)	Cerebral hemisphere	59 (14; 24%)
	Non-temporal	18 (3; 17%)
	Temporal	39 (11; 28%)
	Cerebellar	5 (2)
	Brain stem	3 (0)
	Spinal	2 (0)
PXA (29)	Cerebral hemisphere	27 (16; 59%)
	Non-temporal	6 (4)
	Temporal	17 (11; 65%)
	Cerebellar	1 (0)[a]
	Diencephalic	1 (0)

Diencephalic tumors include chiasmic/hypothalamic, thalamic and pineal region lesions

PA I pilocytic astrocytoma, *GG* ganglioglioma WHO grade I and III, *PXA* pleomorphic xanthoastrocytoma and pleomorphic xanthoastrocytoma with anaplasia

[a] One additional case had a three bp insertion resulting in BRAF p.T599_V600insT

were observed in PXA II (42/64; 66%), aPXA (15/23; 65%), GG I (14/77; 18%), GG III (3/6) and PA I (9/97; 9%). In other types of gliomas, $BRAF^{V600E}$ mutations were less common and restricted to GBM (2/115; 2%), A III (2/58; 3%), O II (1/64; 2%) gcGBM (1/15; 7%), secGBM (1/18; 6%) and GS (1/16; 6%). One instance of $BRAF^{V600E}$ mutation each was identified among the few available cases of gliomatosis cerebri ($N = 5$) and SEGA ($N = 3$). All other investigated nervous system tumors lacked *BRAF* codon 600 alterations.

For lesions with frequent *BRAF* mutations and sufficiently high case numbers, an analysis of mutation frequency by patient age was performed (Fig. 2). Student's *t* test was used to calculate statistically significant differences of mean age. No significant differences were seen for PA I, GG I and PXA II. For aPXA, $BRAF^{V600E}$ mutation is highly significantly associated with younger patient age; patients with *BRAF*-mutant tumors had a mean age of 18 years while patients with *BRAF*-wild-type tumors had a mean age of 38 years ($p = 0.001$). In fact, all 10 pediatric aPXA had $BRAF^{V600E}$ mutations (100%), while the mutation frequency in adult patients was merely 38% (Table 1). Likewise, *BRAF* mutations in GBM and A III of this series all occurred in pediatric patients, with 2/36 pediatric GBM and 2/6 pediatric A III carrying $BRAF^{V600E}$ mutations. No association between *BRAF* mutation and gender was observed (data not shown).

Data on $BRAF^{V600E}$ status and tumor location of PA I, GG and PXA and is given in Table 2. A strong association of tumor location and $BRAF^{V600E}$ mutation was observed in PA I. Eight of 41 (20%) extra-cerebellar PA I were $BRAF^{V600E}$-mutant, whereas only 1 of 53 (2%) cerebellar PA I had this alteration ($p = 0.009$ via Fisher's exact test). Among extra-cerebellar PA I, highest rates were observed in diencephalic tumors with 4 of 12 (33%) of tumors being $BRAF^{V600E}$-mutant. For GG, $BRAF^{V600E}$ mutations were more frequent in temporal location (11/39; 28%), but no significant differences were observed compared to non-temporal supratentorial tumors (3/18; 17%, $p = 0.51$). Location data of PXA was only available for 29 cases. Among these, an association of location and mutation frequency was not evident.

Additional clinical and molecular data of the six adult cases with $BRAF^{V600E}$ mutation not diagnosed as GG, PXA or PA I are summarized in Table 3. The location was recorded in three cases, all of which affected the temporal

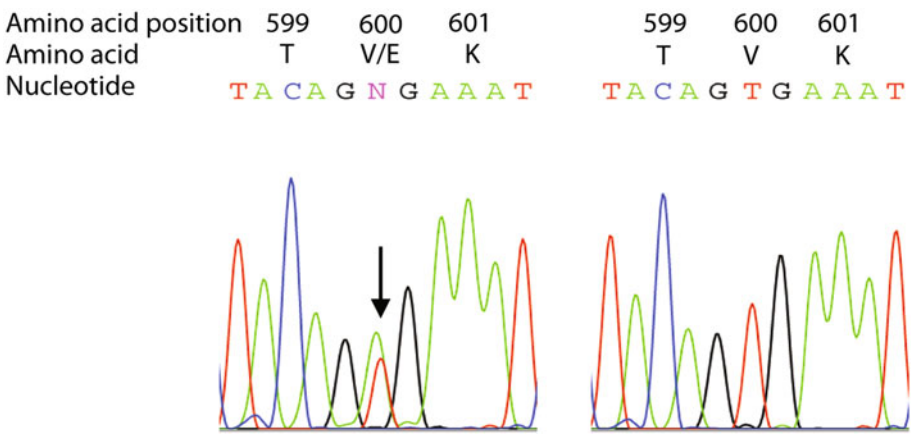

Fig. 1 Illustration of $BRAF^{V600E}$ (*left*, indicated by *arrow*) and BRAF wild-type (*right*) sequence. Note the coexistence of wild-type and mutant sequence in the $BRAF^{V600E}$ mutant sample, representing a heterozygous (monoallelic) mutation

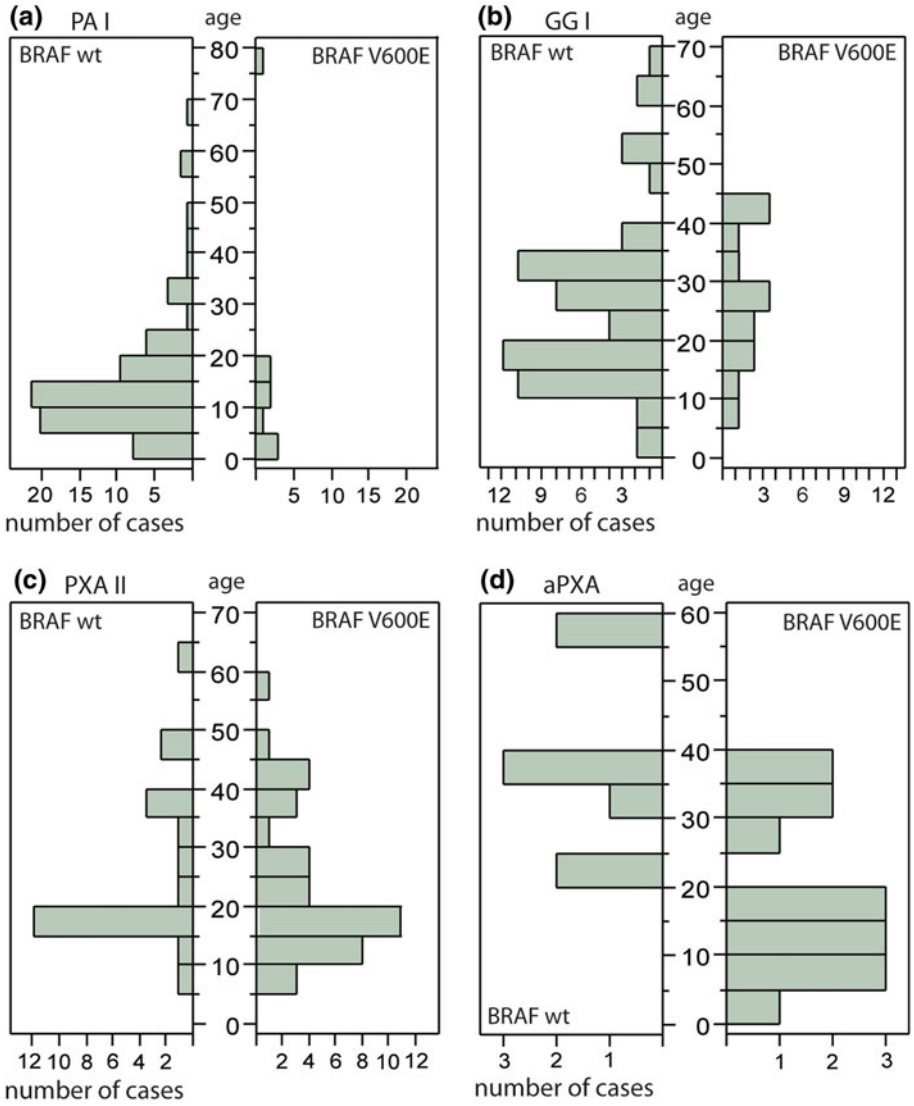

Fig. 2 *BRAF*^{V600E} mutation in relation to age. No significantly different age distribution was observed for pilocytic astrocytoma (**a**), ganglioglioma WHO grade I (**b**) and pleomorphic xanthoastrocytoma WHO grade II (**c**). For pleomorphic xanthoastrocytoma with anaplasia the presence of *BRAF*^{V600E} was significantly associated with younger patient age (**d**; $p = 0.001$)

Table 3 Additional clinical and molecular data of 6 adult patients with gliomas carrying *BRAF*^{V600E} mutations

Histological diagnosis	Age	Gender	Location	Clinical data	Additional molecular data
gcGBM	22	f	NA	Cystic lesion with adjacent tumor nodule, progressed from A II	NA
SEGA	59	m	Temporal left	No other features of tuberous sclerosis, later developed E II in same location	NA
secGBM	46	f	NA	Progressed from A II	*IDH1/2* wt, *p53* wt
O II	39	m	Temporal left	History of seizures, hippocampus and temporal lobe with gliosis	*IDH1/2* wt, no LOH 1p/19q
Gliosarcoma	32	NA	NA	NA	*p53* wt, no LOH 1p/19q, *IDH1* wt
Gliomatosis cerebri	77	f	Temporal left with spread in frontal and occipital lobe	Only stereotactic biopsy taken, histologically A II	NA

gcGBM giant cell glioblastoma, *SEGA* subependymal giant cell astrocytoma, *secGBM* secondary glioblastoma, *O II* oligodendroglioma WHO II, *A II* diffuse astrocytoma WHO II, *E II* ependymoma WHO II, *gen* gender, *NA* not available, *IDH* isocitrate dehydrogenase, *wt* wild type

lobe. Of note, the gcGBM occurred in a 22-year-old patient, presented as a cystic lesion with adjacent tumor nodule and reportedly had progressed from a WHO grade II astrocytoma not diagnosed in any of the participating institutions. The SEGA case presented in a 59-year-old patient, without other evidence of tuberous sclerosis and with the unusual occurrence of a WHO grade II ependymoma at the same tumor location 19 months after resection. The *BRAF*^{V600E} mutant O II lacked isocitrate dehydrogenase 1/2 (*IDH1/2*) gene mutation and loss of heterozygosity of 1p/19q, both molecular aberrations that are highly characteristic for oligodendroglial tumors.

Discussion

Recent data from a phase I clinical trial with the specific inhibitor PLX4032 in patients with metastasized *BRAF*^{V600E} mutant malignant melanoma nourishes the hope that B*RAF*^{V600E} mutation may become a prime target of cancer therapy in the near future [10]. The present study aimed at identifying subsets of nervous system tumors carrying this specific genetic alteration that might be feasible targets for future clinical trials. We demonstrate that two-thirds of PXA, including classical (WHO grade II) tumors and PXA with anaplasia, are characterized by this specific *BRAF* point mutation. This corroborates recent observations in smaller tumor series that demonstrated *BRAF*^{V600E} mutations in few cases of PXA [6, 12, 30]. PXA is a relatively rare tumor entity, accounting for less than 1% of all astrocytic tumors. It typically affects children and young adults and is mostly located in the superficial cortex often extending into the adjacent leptomeninges. It has relatively distinct morphological features, namely pleomorphic and lipidized astrocytic cells, eosinophilic granular bodies, a variably dense reticulin network and prominent perivascular lymphocytic infiltrates [26]. The most frequent genetic alteration of PXA described to date is a homozygous deletion of 9p21.3, including the tumor suppressor gene *CDKN2A* (p16^{INK4a}), which was detected in 6 of 10 (60%) tumors investigated by array-based comparative genomic hybridization [38]. There may well be a functional connection between *CDKN2A* deletion and the high *BRAF* mutation rate detected in this study. It has previously been demonstrated that *BRAF*^{V600E} mutation results in an induction of p16^{INK4a} and that this leads to senescence in human melanocytic nevi [28]. Moreover, expression of activated BRAF alone is not sufficient for gliomagenesis in a mouse model, but the combination of activated BRAF with *CDKN2A* loss is transforming [33]. Thus, it seems possible that in human PXA, *BRAF*^{V600E} induced senescence may be circumvented by concomitant homozygous deletion of *CDKN2A*. A similar connection may exist for other pediatric malignant astrocytomas where 5 of 7 *BRAF*^{V600E} mutated tumors have been shown to carry concomitant homozygous deletions of *CDKN2A* [34]. In one PXA II and two PA I of this series, we observed a different type of *BRAF* mutation resulting in an insertion of threonine between amino acid position 599 and 600 of BRAF. Analogous insertions have previously been described in PA I as well as one tubular adenocarcinoma of the pancreas and were shown to mimic the activating *BRAF*^{V600E} mutation functionally [7, 20, 24].

GG was the second most frequently mutated CNS tumor entity. In our cohort, the *BRAF* mutation frequency was slightly lower than what was expected from a previous investigation of pediatric GG in which 9 of 18 GG (50%) harbored *BRAF*^{V600E} mutations [6]. Still, our data of a large cohort demonstrate that this genetic aberration is moderately frequent ($\sim$20%) in this tumor type and is also found in adult GG. In addition, we show that GG III may have an increased rate of mutations with 3 of 6 tumors in our series carrying mutations. PXA and GG show widely overlapping clinical and histological features and diagnostic distinction may occasionally be difficult. Interestingly, there is ample evidence of transitional tumors with either focal or intermixed features of both PXA and GG [13, 22, 25, 31, 35, 37]. Thus, it seems possible that a subgroup of GG is characterized by *BRAF*^{V600E} mutation and may be more closely related to PXA. Future morphological studies on GG with *BRAF* mutations may clarify this issue. Intriguingly, a fraction of around 10% of GG is also characterized by a homozygous deletion of 9p21 including *CDKN2A,* further indicating a possible relation to PXA [6].

BRAF fusion is the main activating alteration of the RAS/RAF/MEK/ERK kinase pathway in PA I [19, 20, 32]. *BRAF*^{V600E} mutations and *BRAF* fusion are expected to represent mutually exclusive events. Several studies have shown *BRAF* fusion to be substantially more frequent in cerebellar than in extra-cerebellar PA I [12, 16, 17, 19, 23]. Our data indicate that contrary to *BRAF* fusion, *BRAF*^{V600E} is significantly more frequent in extra-cerebellar PA I ($\sim$20%) than in cerebellar tumors ($\sim$2%). While the reason for this is not clear, this observation may have clinical consequences concerning future patient stratification. While most PA I are curable through surgery, outcome may be worse when neuroanatomic realities preclude complete surgical resection as may frequently be the case for deep-seated midline tumors of the diencephalon and brain stem [3]. We here demonstrate that extra-cerebellar PA I and especially diencephalic tumors have frequent *BRAF*^{V600E} mutations and thus represent feasible targets for future clinical trials targeting this specific alteration. The association of *BRAF*^{V600E} and tumor location may further in part explain our slightly higher *BRAF*^{V600E} mutation rate compared to previous reports

[12, 17, 19, 32, 40] as a relatively high proportion of extra-cerebellar PA I were analyzed in this study.

All other investigated CNS tumor entities were characterized by low *BRAF* codon 15 mutation frequencies or completely lacked this alteration. As reported before, our data corroborate that $BRAF^{V600E}$ mutation is rare or absent in adult high-grade glioma [2, 8, 14, 18, 21]. Furthermore, no *BRAF* codon 600 alteration was detected in tumors of the meninges and the PNS. As in a previous study, no *BRAF* mutations were detected among 58 pituitary adenomas [9]. A recent study detected $BRAF^{V600E}$ mutations in 3/3 unusual cases of AT/RT [6]; all three tumors had evolved from lower grade lesions, one from a GG, two from PXA II, two of these cases have been described in detail elsewhere [1, 4]. In line with additional cases investigated by Dougherty and colleagues [6], no *BRAF* codon 600 hot-spot mutations were observed in 14 AT/RT that had typical clinical courses.

As further discussed below, younger patient age is associated with *BRAF* mutation in certain tumor types. Even in pediatric tumors, *BRAF* mutations were detected almost exclusively in PXA, GG and PA I. The only exceptions were pediatric GBM and A III, with 2 of 36 GBM and 2/6 A III showing a $BRAF^{V600E}$ mutation. This is in line with a recent report on high $BRAF^{V600E}$ mutation rates in pediatric high-grade glioma [34]. Of note, in the almost 300 remaining pediatric brain tumors in our series the $BRAF^{V600E}$ mutation was absent. A further observation corroborating the role of patient age and *BRAF* mutation status comes from our cohort of aPXA patients, in which all pediatric cases had the V600E mutation, whereas adult patients harbored this mutation in only 38% of the cases. The reason for this is not clear, although it is possible that the adult group may have included other brain tumors typically occurring at older age, with sometimes strongly overlapping morphology, especially GBM, gcGBM or GS.

Previous investigations have observed infrequent mutations at the codon 600 hot-spot region of *BRAF* in adult gliomas, notably in 1 of 12 O II and 1 of 14 GS [18, 21]. We also detected 6 $BRAF^{V600E}$ mutant adult gliomas of diverse histologies. As listed in Table 3, the *BRAF* mutated O II, gcGBM and SEGA demonstrated somewhat unusual clinical and molecular characteristics and some of these lesions may well represent unrecognized PXA. To resolve this issue, future studies should follow the clinical course of adult *BRAF*-mutant glioma patients to see if these lesions behave more like a PXA than a malignant (WHO grade III or IV) glioma.

The high mutation rates in PXA, GG and extra-cerebellar PA I implicate $BRAF^{V600E}$ mutation as a valuable diagnostic marker. Detection of a $BRAF^{V600E}$ mutation in a pleomorphic glioma should prompt consideration of PXA. In case of a high-grade glioma with an unusually benign clinical course $BRAF^{V600E}$ mutation may indicate misclassification of a PXA or GG. Further, $BRAF^{V600E}$ may aid in the differentiation of extra-cerebellar PA I and diffuse astrocytoma WHO grade II as has previously been demonstrated for *BRAF* fusion [23]. Caution has to be taken though, as $BRAF^{V600E}$ has been detected in several cases of pediatric diffuse astrocytomas WHO grade II [34] and is likely less tumor type specific than *BRAF* fusion.

In summary, we demonstrate high mutation frequencies of $BRAF^{V600E}$ in distinct subtypes of brain tumors: most notably, the majority of PXA are characterized by this specific genetic alteration. The high mutation frequencies in PXA, GG and extra-cerebellar PA I make $BRAF^{V600E}$ alteration a valuable diagnostic marker for these rare tumor entities. Following PA I, PXA is the next tumor entity predominantly occurring in children and young adults that is characterized by very frequent alterations in the RAS/RAF/MEK/ERK kinase pathway. Further analysis of PXA without $BRAF^{V600E}$ will clarify whether the RAS/RAF/MEK/ERK kinase pathway is activated by other means in the remaining tumors and whether PXA represents a "single pathway disease" as has been postulated for PA I. Although PXA, GG and extra-cerebellar PA I constitute rare brain tumor types, future clinical trials should address whether $BRAF^{V600E}$ mutant tumor patients will benefit from $BRAF^{V600E}$-directed targeted therapies.

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