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RESEARCH ARTICLE

KIAA1549-BRAF Fusions and IDH Mutations Can Coexist in Diffuse Gliomas of Adults

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Keywords
1p19q loss, BRAF mutation, deregulation of the Ras-RAF-ERK signaling pathway, diffuse gliomas, IDH1 mutation, KIAA1549/BRAF fusion gene.

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Abstract

KIAA1549-BRAF fusion gene and isocitrate dehydrogenase (IDH) mutations are considered two mutually exclusive genetic events in pilocytic astrocytomas and diffuse gliomas, respectively. We investigated the presence of the *KIAA1549-BRAF* fusion gene in conjunction with IDH mutations and 1p/19q loss in 185 adult diffuse gliomas. Moreover $BRAF^{v600E}$ mutation was also screened. The *KIAA1549-BRAF* fusion gene was evaluated by reverse-transcription polymerase chain reaction (RT-PCR) and sequencing. We found IDH mutations in 125 out 175 cases (71.4%). There were *KIAA1549-BRAF* fusion gene in 17 out of 180 (9.4%) cases and $BRAF^{v600E}$ in 2 out of 133 (1.5%) cases. In 11 of these 17 cases, both IDH mutations and the *KIAA1549-BRAF* fusion were present, as independent molecular events. Moreover, 6 of 17 cases showed co-presence of 1p/19q loss, IDH mutations and *KIAA1549-BRAF* fusion. Among the 17 cases with *KIAA1549-BRAF* fusion gene 15 (88.2%) were oligodendroglial neoplasms. Similarly, the two cases with $BRAF^{v600E}$ mutation were both oligodendroglioma and one had IDH mutations and 1p/19q co-deletion. Our results suggest that in a small fraction of diffuse gliomas, *KIAA1549-BRAF* fusion gene and $BRAF^{v600E}$ mutation may be responsible for deregulation of the Ras-RAF-ERK signaling pathway. Such alterations are more frequent in oligodendroglial neoplasm and may be co-present with IDH mutations and 1p/19q loss.

INTRODUCTION

Mutations in the gene encoding human cytosolic NADP+-dependent isocitrate dehydrogenase (IDH) have been reported to be very frequent, approaching 70%–80% in World Health Organization (WHO) grades II and III diffuse gliomas such as astrocytomas, oligodendrogliomas and oligoastrocytoma (21, 22). Moreover, the occurrence of *IDH1* mutations in WHO grade IV glioblastomas (GBMs) identify such lesions as "secondary" GBM in contrast to "primary" GBM lacking such molecular alterations (14). On the other hand, the WHO grade I pilocytic astrocytomas (PAs), while showing absent or rare *IDH* mutations, frequently disclose a tandem duplication at 7q34, resulting in the fusion of *BRAF* and *KIAA1549* genes, and the production of a chimeric protein with constitutive BRAF activity. This event results in the activation of the extracellular-signal-regulated kinases (ERK)/mitogen-activated protein kinase (MAPK) pathway and promotes G2/M transition in the cell cycle. These two events have so far been considered as mutually exclusive and molecular analysis of these two genetic alterations has been used as a sensitive and highly specific method of distinguishing PAs from diffuse gliomas (2, 5, 9, 10, 12, 15, 18). However, the concept that *IDH* mutations and *BRAF-KIAA1549* fusion gene are mutually exclusive molecular events is derived from a large study based exclusively on fluorescence *in situ* hybridization (FISH) (11). In addition, most of the tumors with *BRAF-KIAA1549* fusion gene were PAs in the pediatric population, whereas tumors with the *IDH* mutation were mostly diffuse gliomas in adults (11). In contrast to adults, *IDH* mutations are rare in pediatric gliomas, irrespective of histological type (1, 16). Therefore, it is possible that the apparent mutual exclusion between presence of the *BRAF-KIAA1549* fusion gene and *IDH* mutation may merely reflect the existence of different classes based on histological subtype (PA vs. diffuse gliomas) and age (children vs. adults). It is not clear whether this mutual exclusion exists in diffuse gliomas in adults. The serendipitous observation of few cases of oligodendrogliomas with the *KIAA1549-BRAF* fusion

gene in adult patients led us to investigate the presence of this alteration in a large series of adult gliomas and to correlate it with *IDH* mutation and 1p/19q loss. Moreover, to further evaluate the involvement of Ras-RAF-ERK signaling pathway in adult diffuse gliomas, mutations for *BRAF* exon 15 ($BRAF^{V600E}$) were screened.

MATERIALS AND METHODS

Patient selection

Samples of gliomas were selected retrospectively on the basis of the availability of fresh frozen material (FF) or formalin-fixed paraffin embedded material (FFPE) from two different institutions: the Mediterranean Neurologic Institute (Neuromed), Pozzilli, Italy, and the Service de Neurologie Hôpital de la Salpêtrière, Paris, France and Laboratoire de Neuropathologie, Hopital del la Timone, Université de la Méditerraneée, Marseille. All cases were histologically classified and graded according to the current WHO classification for tumors of the central nervous sytem (CNS) by KM, DFB and FG (13).

Isolation of DNA ad RNA

DNA was extracted from FF or FFPE tissue specimens with the QIAamp DNA Mini Kit, as described by the manufacturer (Qiagen S.A., Courtaboeuf, France)

Total RNA was extracted from frozen tumor samples by RNeasy Lipid Tissue Mini Kit (Qiagen S.A.) and from FFPE tissue specimens by "RecoverAll total nucleic acid isolation (AMBION)." RNA quality was determined using Agilent 2100 Bioanalyser (Agilent Technologies, Massy, France).

DNA and RNA concentrations were quantified using the Nanodrop ND-1000 UV-Vis spectrophotometer (Labtech France, Palaiseau, France). Final products were stored at –40°C until use.

BRAF-KIAA1549 fusion gene detection by RT-PCR and sequencing

Reverse-transcription polymerase chain reaction (RT-PCR) was performed on 1 or 3 μg of total RNA (respectively for FF or FFPE samples) using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The integrity of the resulting cDNAs from FFPE tumor samples was checked by amplifying the wild-type locus of the *BRAF* gene. All cDNAs were then submitted to PCR with specific pairs of primers flanking the fusion point between the *KIAA1549* (in exon 15 or 16) and *BRAF* (in exon 9 or 11) genes as described by Jones *et al* (9). The purified PCR products were then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Courtaboeuf, France) with forward or reverse primer used to perform the PCR. Sequencing was performed using the ABI 3730 DNA analyser (Applied Biosystems, Foster City, CA, USA) and analyzed with Chromas Lite software (Technelysium Pty Ltd, Queensland, Australia). The sequences of primers used were as follows: *KIAA1549* exon 15: 5′-CGG AAA CAC CAG GTC AAC GG-3″; *KIAA1549* exon 16: 5′-AAA CAG CAC CCC TTC CCA GG-3′; *BRAF* exon 9: 5′-CTC CAT CAC CAC GAA ATC CTT G-3′; *BRAF* exon 11: 5′-GTT CCA AAT GAT CCA GAT CCA TTC-3′.

IDH and $BRAF^{v600E}$ mutation analysis

We used 50 ng of total DNA to amplify a 122 bp PCR product for the *IDH1* gene and a 115 bp PCR product for the *IDH2* gene. The primers used were as follows: *IDH1* exon 4, 5′-CGG TCT TCA GAG AAG CCA TT-3′ (sense) and 5′-CAC ATT ATT GCC AAC ATG AC-3′ (antisense); *IDH2* exon 4, 5′-AGC CCA TCA TCT GCA AAA AC-3′ (sense) and 5-CAG TGG ATC CCC TCT CCA C-3′ (antisense); *BRAF* exon 15, 5′- TCA TAA TGC TTG CTC TGA TAG GA-3′ (sense) and 5′-GGC CAA AAA TTT AAT CAG TGG A-3′ (antisense). The PCR products were then purified and sequenced as described in the previous paragraph.

Assessment for 1p/19q deletion

Assessment of loss of 1p/19q was performed using two different methods:

(i) Microsatellite analysis with multiplex PCR for highly polymorphic microsatellite repeated sequences on 1p and 19q, detected by capillary electrophoreses. After electrophoresis, data were collected with the Gene Scan program for fragment analysis (ABI Prism Applied Biosystems, Foster City, CA, USA; Perkin Elmer, Boston, MA, USA). Allelic imbalance was evaluated by comparing PCR products from tumor and normal DNA in neighboring lanes. The ratios of the peak heights of the two alleles were calculated in blood (N1/N2) and tumor (T1/T2) DNA. Allelic imbalance resulted from the ratio of normal to tumor signal (N1/N2 over T1/T2). LOH values less than or equal to 0.5 indicate the loss of the longer allele in the tumor, whereas LOH values greater than or equal to 1.5 indicate the loss of the shorter allele (4).

(ii) Comparative genomic hybridization (CGH) array based on 4500 sequence-validated bacterial artificial chromosomes (BACs) (Integragen, Paris, France), performed as previously described (8).

RESULTS

Patient characteristics

We selected 185 diffuse gliomas occurring in patients aged >20 years (104 males/81 females; median age, 42 years; range, 20–84 years). These tumors were classified as follows: 18 astrocytomas grade II; 17 anaplastic astrocytomas grade III; 36 oligodendrogliomas grade II; 38 anaplastic oligodendrogliomas grade III; 23 oligoastrocytomas grade II; 35 anaplastic oligoastrocytomas grade III; and 18 GBMs grade IV. FF material was available for 155 tumors, while only FFPE material was available for the remaining 30 cases (Table 1). In addition, we selected 31 nondiffuse gliomas (13 PAs and 18 gangliogliomas).

Molecular profiles

In diffuse gliomas, sequencing revealed *IDH* mutations in 125 of 175 (71.4%) cases. The distribution of mutations was the following: 107 (85.6%) $IDH1^{R132H}$, 5 (4%) $IDH1^{R132G}$, 3 (2.4%) $IDH1R^{132C}$, 2 (1.6%) $IDH1^{R132L}$, 1 (0.8%) $IDH1^{R132S}$, 6 (4.8%) $IDH2^{R172K}$ and 1(0.8%) $IDH2^{R172S}$. Co-deletion of 1p/19q, evaluated by microsatellite analysis in 26 cases and CGH in 147 cases, was detected in 53 out of 173 cases (30.6%).

Table 1. Clinical and pathological data and tissue type used in molecular analysis. Abbreviations: FF = fresh frozen; FFPE = formalin-fixed paraffin-embedded; WHO = World Health Organization.

Histology*	Median age (years)	Location		FF	FFPE	Total (%)
		Subtentorial	Supratentorial			
Nondiffuse gliomas (n = 31)						
Pilocytic astrocytoma	26	7	6	12	1	13 (41.9)
Ganglioglioma	38	0	18	15	3	18 (58)
Diffuse gliomas (n = 185)						
Astrocytoma II	37	0	18	14	4	18 (9.7)
Astrocytoma III	45	0	17	13	4	17 (9.1)
Oligodendroglioma II	41	0	36	29	7	36 (19.4)
Oligodendroglioma III	49	0	38	31	7	38 (20.5)
Oligoastrocytoma II	34	0	23	21	2	23 (12.4)
Oligoastrocytoma III	43	0	35	35	0	35 (18.9)
Glioblastoma grade IV	58	0	18	16	2	18 (9.7)

*Roman numerals refer to WHO histological grade.

RT-PCR from RNA extracted from FF or FFPE samples followed by amplicon sequencing showed presence of the $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene in 17 of 180 (9.4%) diffuse gliomas (Figure 1; Table 2). The frequency of this alteration in the series was as follows: one astrocytoma grade II; four oligodendrogliomas grade II; eight oligodendrogliomas grade III; two oligoastrocytomas grade II; one oligoastrocytoma grade III; and one GBM grade IV. Of these 17 cases with the $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene, 10 showed copresence of $IDH1^{R132H}$ mutations (58.8%) (Figure 2) and in 1 out 17 cases, the presence of the *KIAA1549-BRAF* fusion gene was associated with $IDH2^{R172K}$ mutation (Table 3). The frequency of *IDH1/2* mutations was similar to that observed in gliomas without the $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene (105 of 158; 66.4%; $P = 0.6$ Fisher's exact test). These results suggest that *IDH* mutations and the presence of the *KIAA1549-BRAF* fusion gene are independent events in adult diffuse gliomas.

The 1p/19q co-deletion was present in 6 of 17 (35.2%) diffuse gliomas with the $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene and in 47 of 159 (29.5%) diffuse gliomas without the fusion gene ($P = 0.36$, Fisher's exact test) (Table 3). Again, these results suggest that 1p/19q co-deletion and the presence of the $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene are not mutually exclusive events. Finally, in 6 out of 162 cases (3.7%), we observed the coexistence of IDH mutations, $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene and 1p/19q co-deletion (Figure 3).

Analysis sequencing revealed $BRAF^{V600E}$ mutation in 2 of 133 (1.5%) cases: an oligodendroglioma (grade II) which was not associated with IDH mutation nor 1p/19q co-deletion and one anaplastic oligodendroglioma (grade III) associated with $IDH1^{R132H}$ mutation and 1p/19q co-deletion. Both these cases lacked $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene.

In contrast to infiltrative diffuse gliomas, none of the adult nondiffuse gliomas had IDH mutations or 1p/19q co-deletion, while the $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene was detected in 8 of 31

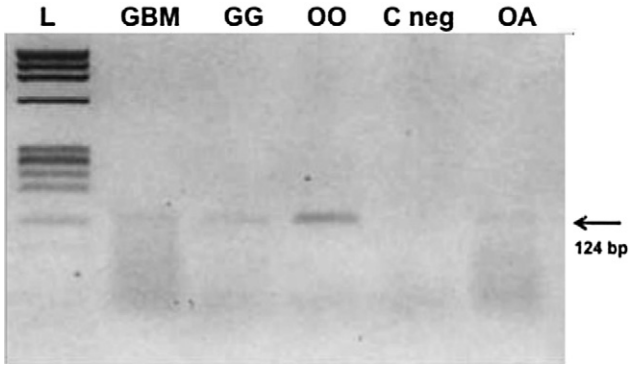

Figure 1. RT-PCR from FFPE tissue showing $KIAA1549^{Ex16}$-$BRAF^{Ex9}$ fusion gene in four gliomas: glioblastoma (GBM), ganglioglioma (GG); oligodendroglioma (OO); oligoastrocytoma (OA); C neg, negative control; L, molecular size marker. RT-PCR = reverse-transcription polymerase chain reaction; FFPE = formalin-fixed paraffin-embedded.

Table 2. Frequency of IDH mutations and KIAA1549-BRAF fusion gene in nondiffuse and diffuse gliomas. Abbreviations: IDH = isocitrate dehydrogenase; WHO = World Health Organization.

Histology*	*IDH* mutation†		*KIAA1549-BRAF* fusion (%)‡
	IDH1 mutation (%)	*IDH2* mutation (%)	
Nondiffuse glioma			
Pilocytic astrocytoma	0	0	6/13 (46.1)
Ganglioglioma	0	0	2/18 (11.1)
Total	0	0	8/31 (25.8)
Diffuse glioma			
Astrocytoma II	14 (8)	0	1 (0.5)
Astrocytoma III	8 (4.5)	1 (0.5)	0
Oligodendroglioma II	24 (13.7)	3 (1.7)	4 (2.2)
Oligodendroglioma III	27 (15.4)	3 (1.7)	8 (4.4)
Oligoastrocytoma II	16 (9.1)	0	2 (1.1)
Oligoastrocytoma III	26 (14.8)	0	1 (0.5)
Glioblastoma IV	3 (1.7)	0	1 (0.5)
Total	118 (67.4)	7 (4)	17 (9.4)

*Roman numerals refer to WHO histological grade.
†175 cases evaluated for IDH mutations.
‡180 cases evaluated for *KIAA1549-BRAF* fusion gene.

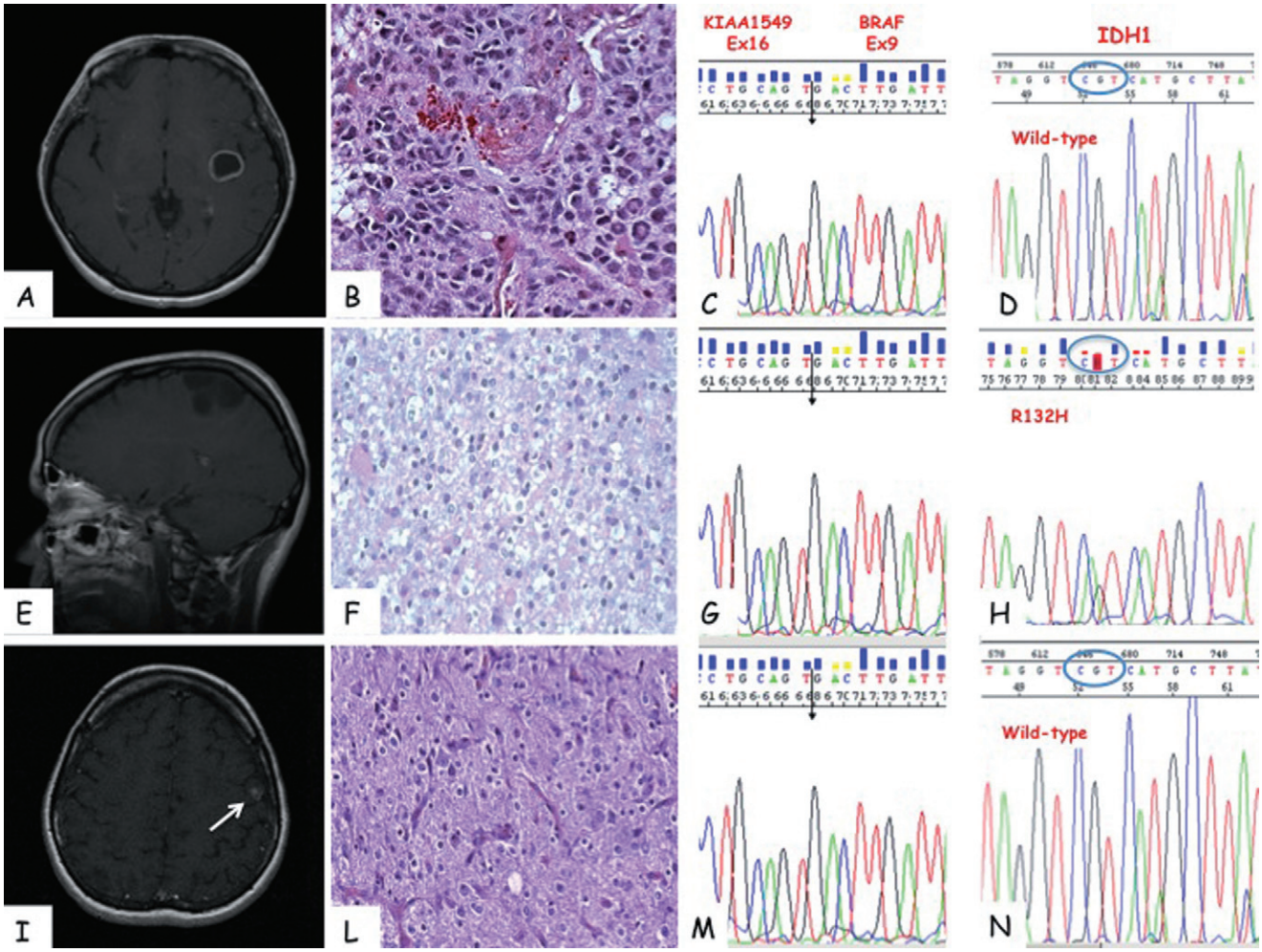

Figure 2. This panel illustrates three cases with *KIAA1549*^{Ex16}-*BRAF*^{Ex9} fusion confirmed by sequencing (C,G,M) and correlates the radiological-histological features with the corresponding sequencing analysis of IDH1: glioblastoma (**A–D**): (**A**) T1-weighted MRI shows an insular lesion with ring-enhancement; (**B**) histological features showing hypercellularity, cellular atypia and vessels proliferation; (**D**) sequencing analysis showing IDH1 gene wild-type; oligoastrocytoma (**E–H**): (**E**) T1-weighted MRI with contrast reveals a hypointense nonenhancing parietal lesion. (**F**) Histological examination shows an oligoastrocytoma grade II with R132H mutation in IDH1 gene (**H**); ganglioglioma (**I–N**): (**I**) T1-weighted MRI shows a small enhancing cortical lesion (arrow) composed of a mixed population of glial and mature ganglion cells (**L**) and IDH1 wild-type gene (**N**).

cases, including 6 of 13 PAs (46.1%) and 2 of 18 gangliogliomas (11.1%) (Table 2).

Clinical and pathological data of diffuse gliomas with *KIAA1549*^{Ex16}-*BRAF*^{Ex9} fusion gene

The clinical data in term of treatment and follow-up of the 17 cases showing the *KIAA1549*^{Ex16}-*BRAF*^{Ex9} fusion gene are summarized in Table 3. All the neoplasms showed standard histological appearance without any features of a pilocytic component. All the patients had adjuvant therapy according to the grade of the neoplasms. There was a single case of primary GBM without IDH mutations with a survival of 8 months after surgery. There were 9 out of 11 (81.8%) patients with IDH mutations who were alive with follow-up, ranging from 21 to 72 months. In contrast, among the four anaplastic oligodendrogliomas without IDH mutations, one patient was alive after 13 months from surgery and three were deceased, with a survival ranging from 8 to 46 months.

DISCUSSION

Until now, the formation of the *KIAA1549-BRAF* fusion gene and *IDH* mutations have been considered to be two genetic events that occur in a mutually exclusive manner in PAs and diffuse gliomas, respectively (11). These two types of neoplasm occur in two different age groups (ie, PAs in children and diffuse gliomas in adults), and most previous studies have investigated either *BRAF* alterations or *IDH* mutations in pediatric or adult populations. Our study is the first to screen for both *IDH* mutations and the *KIAA1549-BRAF* fusion gene in a large series of gliomas in adults.

Table 3. Clinical and pathological data of diffuse gliomas with *KIAA1549*^{Ex16}-*BRAF*^{Ex9} fusion gene and their association with IDH mutations and/or 1p/19q loss. Abbreviations: A = astrocytoma; GBM = glioblastoma; IDH = isocitrate dehydrogenase; Mut = mutant; NA = not analyzed; OA = oligoastrocytoma; OO = oligodendroglioma; WHO = World Health Organization; WT = wild type.

Case	Sex	Age (years)	Diagnosis*	Location	*KIAA1549*^{Ex16}-*BRAF*^{Ex9}	*IDH1* fusion	*IDH2*	1p19q loss	Follow-up (months)
1	Female	52	GBM	Insular	Yes	WT	NA	NA	8 (dead)
2	Male	50	OOII	Temporal	Yes	Mut†	NA	Yes	72 (alive)
3	Female	32	OA II	Parietal	Yes	Mut†	NA	Yes	70 (alive)
4	Male	45	OO III	Frontal	Yes	Mut†	NA	NA	70 (alive)
5	Female	50	OO III	Multifocal	Yes	Mut†	NA	Yes	48 (alive)
6	Female	35	OA II	Frontal	Yes	Mut†	NA	Yes	47 (alive)
7	Male	34	OO II	Frontal	Yes	Mut†	NA	Yes	36 (alive)
8	Female	53	OO III	Frontal	Yes	NA	NA	NA	24 (dead)
9	Female	33	OA III	Hemispheric	Yes	Mut†	WT	No	45 (alive)
10	Female	32	A II	Hemispheric	Yes	Mut†	WT	No	48 (dead)
11	Female	53	OO II	Hemispheric	Yes	Mut†	WT	No	21 (alive)
12	Male	46	OO II	Hemispheric	Yes	Mut†	WT	Yes	29 (alive)
13	Female	43	OO III	Hemispheric	Yes	WT	Mut‡	No	19 (dead)
14	Male	44	OO III	Frontal	Yes	WT	WT	No	16 (dead)
15	Male	43	OO III	Temporal-occipital	Yes	WT	WT	No	26 (dead)
16	Male	41	OO III	Temporal	Yes	WT	WT	No	13 (alive)
17	Female	73	OO III	Hemispheric	Yes	WT	WT	No	46 (dead)

*Roman numerals refer to WHO histological grade.
†*IDH1*^{R132H}
‡*IDH2*^{R172K}

Tandem duplication at 7q34 leading to a fusion between *KIAA1549* and *BRAF* with consequent activation of the MAPK pathway has been detected in about 66% of PAs (2, 5, 9, 10, 12, 15, 18), but has also been observed occasionally in other low-grade gliomas. Various studies based on microarray copy-number analyses have identified gains at 7q34 in 7 of 54 (13%) grade II astrocytomas and 5 of 96 (5%) mixed astrocytic/oligodendroglial tumors (2, 9, 10, 12, 15, 18). Additionally, 2 of 5 nonpilocytic low-grade diffuse astrocytomas that had not been tested for the 7q34 gain were found to carry the *KIAA1549-BRAF* fusion by PCR and sequencing analyses (5, 19). In our series of nondiffuse gliomas, we detected the *KIAA1549-BRAF* fusion gene in 46% of PAs. The lower percentage we found, compared with previous studies, may be related to the older age of the patients selected and the higher frequency of supratentorial location (6, 12). Moreover, 2 of 18 gangliogliomas (11%) also had the *KIAA1549-BRAF* fusion gene. The occurrence of the *KIAA1549-BRAF* fusion gene in gangliogliomas and low-grade glioneuronal tumors has been previously reported (12). None of the nondiffuse gliomas showed mutation of the *IDH* genes.

Figure 3. This Venn diagram visualizes the frequency of the four molecular alterations and their coexistence in diffuse gliomas of adults. IDH = isocitrate dehydrogenase.

In the series of diffuse gliomas, we found, as expected, frequent *IDH* mutations (125 of 175; 71.4%). However, the most interesting finding was the detection of the *KIAA1549-BRAF* fusion in 17 cases (9.4%). Noteworthy, 15 out 17 (88.2%) diffuse gliomas with such alteration were oligodendroglial neoplasms (Table 3). Nevertheless, the frequency of *IDH* mutations was similar to that observed in gliomas without the *KIAA1549*^{Ex16}-*BRAF*^{Ex9} fusion gene, suggesting that *IDH* mutations and the presence of the *KIAA1549-BRAF* fusion gene are independent events in adult diffuse gliomas.

This finding contrasts with the notion that *IDH* mutation and *BRAF-KIAA1549* fusion are mutually exclusive. This concept is largely derived from a study by Korshunov *et al* (11) that is based exclusively on FISH analysis in both pediatric and adult gliomas. In this study, gain of 7q34 was detected in 83 of 120 tumors, with 52 PAs and 31 diffuse gliomas of WHO grade II exhibiting more than two signals each for *BRAF* and *KIAA1549* in tumor cells. These data demonstrate that gain of chromosomal material on 7q34 is

common in both PAs and diffuse gliomas. However, according to these authors, the nature of gains in the *BRAF* region differs between PAs and diffuse gliomas on the basis that they were able to detect by FISH the fusion of *BRAF* and *KIAA1549* predominantly in PAs, and only in a single instance in a diffuse grade II glioma. A major criticism of this study is that although FISH analysis is a powerful method to detect chromosomal alteration and particularly chromosomal gain or loss, it may be subject to laboratory variability when dealing with more subtle alterations such as fusion genes (7). In FISH analysis, the probes used can detect genomic duplications involving the 3′ region of BRAF (the same region involved in the KIAA1549-BRAF fusion), but are not specific for this rearrangement. Moreover, a threshold of 15% is established by evaluation of normal tissue and is used as a minimum for determining BRAF duplication. Cases showing a lower percentage of positive cells are considered not duplicate (20). On the basis of these considerations, our study based on detection of the fusion gene by RT-PCR assay followed by direct sequencing of the PCR product has allowed to detect such rare molecular alteration in a large cohort of adult diffuse gliomas.

Another interesting and previously unreported finding of the present study was the occurrence of 1p/19q loss in 6 of 10 cases in which *IDH* mutations and *KIAA1549-BRAF* fusion were copresent. All these "triple-positive" cases were oligodendroglial tumors (five oligodendrogliomas and one oligoastrocytoma) (Figure 3). In this study, we also found $BRAF^{V600E}$ mutation in two out of 133 diffuse gliomas (1.5%). They both were oligodendrogliomas, and in one of them such mutation was associated with IDH mutations and 1p/19q loss. This is consistent with the results of a previous study on 162 low-grade diffuse gliomas, which showed that only one oligodendroglioma had a $BRAF^{V600E}$ mutation (17).

Recent studies have found a high frequency of CIC mutations in oligodendrogliomas in associations with IDH mutations and 1p/19 anomalies (3, 23). The significance that these alterations, together with KIAA1549-BRAF fusion gene and $BRAF^{V600E}$, coexist more frequently in oligodendroglial neoplasms needs further investigations.

The clinical significance of *BRAF* alterations in diffuse gliomas remains unclear. The observed deregulation of the Ras-RAF-ERK signaling pathway in nonpilocytic gliomas is attributed to its upstream positive regulators, including epidermal growth factor receptor and platelet-derived growth factor receptor, which are known to be highly active in the majority of diffuse gliomas. Our results, however, suggest that in a small percentage of diffuse gliomas, such deregulation might be related to 7q34 rearrangements, resulting in a novel in-frame *KIAA1549-BRAF* fusion gene like that found in PAs. In addition, we demonstrate that such alterations can be associated with *IDH* mutations and 1p/19q loss.

Future studies should expand on these observations and continue to define the heterogeneous genetic and biological features of adult gliomas and the role of *BRAF* alterations in the pathogenesis and clinical behavior of these tumors.

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