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Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas.

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Abstract

Somatic mutations in the IDH1 gene encoding cytosolic NADP+-dependent isocitrate dehydrogenase have been shown in the majority of astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III. IDH2 encoding mitochondrial NADP+-dependent isocitrate dehydrogenase is also mutated in these tumors, albeit at much lower frequencies. Preliminary data suggest an importance of IDH1 mutation for prognosis showing that patients with anaplastic astrocytomas, oligodendrogliomas and oligoastrocytomas harboring IDH1 mutations seem to fare much better than patients without this mutation in their tumors. To determine mutation types and their frequencies, we examined 1,010 diffuse gliomas. We detected 716 IDH1 mutations and 31 IDH2 mutations. We found 165 IDH1 (72.7%) and 2 IDH2 mutations (0.9%) in 227 diffuse astrocytomas WHO grade II, 146 IDH1 (64.0%) and 2 IDH2 mutations (0.9%) in 228 anaplastic astrocytomas WHO grade III, 105 IDH1 (82.0%) and 6 IDH2 mutations (4.7%) in 128 oligodendrogliomas WHO grade II, 121 IDH1 (69.5%) and 9 IDH2 mutations (5.2%) in 174 anaplastic oligodendrogliomas WHO grade III, 62 IDH1 (81.6%) and 1 IDH2 mutations (1.3%) in 76 oligoastrocytomas WHO grade II and 117 IDH1 (66.1%) and 11 IDH2 mutations (6.2%) in 177 anaplastic oligoastrocytomas WHO grade III. We report on an inverse association of IDH1 and IDH2 mutations in these gliomas and a non-random distribution of the mutation types within the tumor entities. IDH1 mutations of the R132C type are strongly associated with astrocytoma, while IDH2 mutations predominantly occur in oligodendroglial tumors. In addition, patients with anaplastic glioma harboring IDH1 mutations were on average 6 years younger than those without these alterations.

Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation, grade and age: A study of 1010 diffuse gliomas

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Abstract

Somatic mutations in the *IDH1* gene encoding cytosolic NADP+ dependent isocitrate dehydrogenase have been shown in the majority of astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III. *IDH2* encoding mitochondrial NADP+ dependent isocitrate dehydrogenase also is mutated in these tumors, albeit at much lower frequencies. Preliminary data suggest an importance of *IDH1* mutation for prognosis showing that patients with anaplastic astrocytomas, oligodendrogliomas and oligoastrocytomas harbouring *IDH1* mutations seem to fare much better than patients without this mutation in their tumors. To determine mutation types and their frequencies, we examined 1010 diffuse gliomas. We detected 716 *IDH1* mutations and 31 *IDH2* mutations. We found 165 *IDH1* (72.7%) and 2 *IDH2* mutations (0.9%) in 227 diffuse astrocytomas WHO grade II, 146 *IDH1* (64.0%) and 2 *IDH2* mutations (0.9%) in 228 anaplastic astrocytomas WHO grade III, 105 *IDH1* (82.0%) and 6 *IDH2* mutations (4.7%) in 128 oligodendrogliomas WHO grade II, 121 *IDH1* (69.5%) and 9 *IDH2* mutations (5.3%) in 174 anaplastic oligodendrogliomas WHO grade III, 62 *IDH1* (81.6%) and 1 *IDH2* mutations (1.3%) in 76 oligoastrocytomas WHO grade II and 117 *IDH1* (66.1%) and 9 *IDH2* mutations (5.1%) in 177 anaplastic oligoastrocytomas WHO grade III. We report on an inverse association of *IDH1* and *IDH2* mutations in these gliomas and a non-random distribution of the mutation types within the tumor entities. *IDH1* mutations of the R132C type are strongly associated with astrocytoma while *IDH2* mutations predominantly occur in oligodendroglial tumors of WHO grade III.

Keywords: *IDH1*, *IDH2*, mutation, glioma, astrocytoma, oligodendroglioma, oligoastrocytoma

Introduction

Extraordinary high rates of spontaneous mutations in the gene encoding cytosolic NADP+ dependent isocitrate dehydrogenase (*IDH1*) have been reported in diffuse gliomas of World Health Organization (WHO) grades II and III of astrocytic and oligodendroglial lineages [1, 8, 17, 21] and in lower frequency mutations in the gene encoding mitochondrial NADP+ dependent isocitrate dehydrogenase (*IDH2*) [21]. In contrast, mutations of *IDH1* are rare in primary glioblastoma [1, 2, 8, 9, 14, 17, 21]. These mutations appear to be of significant importance for survival of patients. Both, patients with anaplastic astrocytoma and glioblastoma show significantly longer overall survival in the presence of *IDH1* or *IDH2* mutations [21]. Analysis of a prospective study demonstrated that absence of *IDH1* mutations in anaplastic astrocytoma, oligoastrocytoma and oligodendroglioma of WHO grade III is a strong indicator for poor prognosis [20].

Isocitrate dehydrogenases catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate. Five genes encoding human isocitrate dehydrogenases have been identified. *IDH1* on 2q33.3 encodes cytosolic NADP+ specific isocitrate dehydrogenase [13]. IDH1 is configured as a homodimer with two enzymatically active sites and most of its activity is detected in the cytosol and in peroxysomes [4]. A major function of IDH1 is believed to be the synthesis of NADPH required for reducing reactions and for lipid synthesis [16]. *IDH2* on 15q26.1 encodes the mitochondrial NADP+ specific isocitrate dehydrogenase [5]. Similar to IDH1, this enzyme functions as a homodimer. Recent findings suggest that the IDH2 may be the main catalyst for the oxidation of isocitrate to α-ketoglutarate in the citric acid cycle [6]. IDH3 is composed of three subunits encoded by *IDH3A* (subunit alpha), on 15q25.1-q25.2 [7], by *IDH3B* (subunit beta) on 20p13 [10] and by *IDH3G* (subunit gamma), on Xq28. IDH3 is a multi-tetrameric enzyme ($2\alpha1\beta1\gamma$) with α–subunits being catalytic and the β- and γ -subunits being believed to be regulatory [15, 19]. IDH3 utilizes NAD+ as a coenzyme. The function of IDH3 in the citric acid cycle is well established.

Glioma-specific mutations in *IDH1* always affected the amino acid arginine in position 132 of the amino acid sequence which belongs to an evolutionary highly conserved region located at the binding site for isocitrate [14]. Mutations in *IDH2* were exclusively detected in arginine at position 172 which is the analogous site to arginine 132 in *IDH1* [21]. Mutations in both, *IDH1* and *IDH2* are heterozygous and of somatic origin. The role of *IDH1* mutations in tumor biology currently is intensely studied. Mutations inactivate enzyme activity [21]. This inactivation is due to impaired substrate affinity, and

moreover, *IDH1* mutations exert a dominant negative effect rendering heterodimers inactive as well [22]. Enzyme deficiency results in depletion of α-ketoglutarate which is required for prolylhydroxylases promoting degradation of hypoxia inducible factor 1α (HIF-1α). Increased HIF-1α levels in gliomas carrying the *IDH1* R132H mutations have been demonstrated [22]. However, there are many other oxygenases which are dependent on α-ketoglutarate and which are involved in different processes such as histone modification or fatty acid metabolism [11]. Therefore, several other tumor relevant mechanisms besides HIF-1α stabilization may result from *IDH1* mutation mediated depletion of α-ketoglutarate.

In order to determine the different types of mutations and their frequencies, we examined 1010 human gliomas for mutations in codons 132 and 172 in the genes for *IDH1* and *IDH2*, respectively. Because high frequencies of *IDH1* mutations have been described only in few glioma subtypes, we focussed in the present study on these entities including WHO grade II and III astrocytomas, oligodendrogliomas and oligoastrocytomas

Material and Methods

Tumor specimens average patient age and sex ratio

DNA samples from human brain tumors diagnosed at the Departments of Neuropathology of the Universities Heidelberg, Bonn, Düsseldorf, Nijmegen, Magdeburg and at the Charité Berlin were analyzed. All tumors were diagnosed and classified according to the WHO classification of tumors of the nervous system [12]. The series consisted of 1010 diffuse gliomas including 227 diffuse astrocytomas WHO grade II (A II), 228 anaplastic astrocytomas WHO grade III (A III), 128 oligodendrogliomas WHO grade II (O II), 174 anaplastic oligodendrogliomas WHO grade III (O III), 76 oligoastrocytomas WHO grade II (OA II) and 177 anaplastic oligoastrocytomas (OA III). The *IDH1* mutation data of 281 patients in this series have been reported in a preceding study [1].The average mean ages and female to male sex ratios were for A II 37 years and 44% to 56%, for A III 42 years and 40% to 60 %, for O II 44 years and 43% to 57%, for O III 49 years and 47% to 53%, for OA II 43 years and 62% to 38% and for OA III 47 years and 45% to 55%.

PCR amplification:

Primer design was based on accession numbers NM_005896 for *IDH1* and NM_002168 for *IDH2* (http://www.ncbi.nlm.nih.gov*)*. A fragment of 129 bp length spanning the sequence encoding the catalytic domain of *IDH1* including codon 132 was amplified using 60 ng each of the sense primer IDH1f CGGTCTTCAGAGAAGCCATT and the antisense primer IDH1r GCAAAATCACATTATTGCCAAC. PCR using standard buffer conditions, 20 ng of DNA and GoTaq DNA Polymerase (Promega, Madison, WI, USA) employed 35 cycles with denaturing at 95° C for 30 sec, annealing at 56° C for 40 sec and extension at 72° C for 50 sec in a total volume of 15 µl.
For confirmation, the sense primer IDH1fc ACCAAATGGCACCATACGA and antisense primer IDH1rc TTCATACCTTGCTTAATGGGTGT generating a 254 bp fragment at the same PCR conditions were employed.
A fragment of 150 bp length spanning the sequence encoding the catalytic domain of *IDH2* including codon 172 was amplified using 60 ng each of the sense primer IDH2f AGCCCATCATCTGCAAAAAC and the antisense primer IDH2r CTAGGCGAGGAGCTCCAGT. PCR using standard buffer conditions, 20 ng of DNA and GoTaq DNA Polymerase (Promega,Madison, USA) employed 35 cycles with

denaturing at 95° C for 30 sec, annealing at 58° C for 40 sec and extension at 72° C for 50 sec in a total volume of 15 µl.

For confirmation, the sense primer IDH2fc GCTGCAGTGGGACCACTATT and antisense primer IDH2rc TGTGGCCTTGTACTGCAGAG generating a 293 bp fragment at the same PCR conditions were employed.

Direct sequencing:

Two µl of the PCR amplification product was subjected sequencing using the BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Twenty-five cycles were performed employing 12 ng of the sense primers IDH1f CGGTCTTCAGAGAAGCCATT or IDH2f CTAGGCGAGGAGCTCCAGT, with denaturing at 95° C for 30 sec, annealing at 56° for 15 sec and extension at 60° C for 240 sec. In case of ambiguous readings, a second round of sequencing analysis was performed using the antisense primer IDH1rc TTCATACCTTGCTTAATGGGTGT or IDH2rc TTCATACCTTGCTTAATGGGTGT and the sequencing reaction conditions as described above. Sequences were determined using the semiautomated sequencer (ABI 3100 Genetic Analyzer, Applied Biosystems) and the Sequence Pilot version 3.1 software (JSI-Medisys, Kippenheim, Germany).

Statistics:

The Fisher exact test was used to examine associations between nominal variables referring to absence or presence of genetic alterations. The relationship of *IDH* mutations with patient age was examined by student t test.

Results and Discussion

We detected 716 *IDH1* mutations and 31 *IDH2* mutations in 1010 patients with astrocytoma, oligodendroglioma or oligoastrocytoma of WHO grades II and III. Only codon 132 of *IDH1* and codon 172 of *IDH2* were affected by mutations. Previous studies did not detect *IDH1* and *IDH2* mutations in constitutional tissues [1, 8, 14, 17, 21]. Therefore we did not analyze corresponding blood samples from the glioma patients included in this study. In the majority of the cases, mutations in codon 132 of *IDH1* and codon 172 of *IDH2* were obvious upon sequencing in forward direction. However, we encountered cases yielding only weak peaks in addition to the signal of the wild type sequence. These cases suspected to carry a mutation were sequenced a second time in reverse direction. Reappearance of an additional signal at the site of suspect was then scored as positive for mutation. Based on direct sequencing, all mutations appeared to be heterozygous meaning that in each tumor with a mutation only one of the two gene copies of *IDH1* or *IDH2,* respectively, was altered.

Type and frequency of IDH1 and IDH2 mutations

The predominant amino acid sequence alteration in *IDH1* was R132H accounting for 92.7% of the detected mutations followed by R132C for 4.1%, R132S for 1.5%, R132G for 1.4% and R132L for 0.2% of all *IDH1* mutations. Type and distribution of the mutations are given in Table 1. We did not detect the R132V mutation reported and illustrated in our previous series [1]. The distribution of mutations in the present series matches well with those of other studies demonstrating the vast majority of *IDH1* mutations being of the R132H type followed by R132C. The discrepancies in literature regarding the low frequencies of R132S, R132G and R132L may be due to differences in sample size and different types of tumors analyzed.

In *IDH2* the distribution of mutation types is less off-centre. R172K made up 65%, R172M amounted to 19% and the previously not described R172W to 16% of all *IDH2* mutations. In contrast to the initial report on *IDH2* mutations, we did not find the R172G exchange. While in several instances we did detect a shallow G peak in nucleotide position 514, we interpreted this signal as artificial and caused by the flanking G nucleotides in positions 511, 512, 515 and 516 of the wild type sequence of *IDH2*. An example for such a signal interpreted as sequencing artefact is shown in Figure 1.

The amino acid sequence of *IDH1* and *IDH2* at the sites of mutations are identical while the nucleotide sequences differ. It is noteworthy that the most frequent mutations in both, *IDH1* and *IDH2* derive from a G to A transition in nucleotide position 395 of *IDH1* and 515 of *IDH2*, respectively.

IDH1 and IDH2 mutations in astrocytoma, oligodendroglioma and oligoastrocytoma

We detected 716 *IDH1* mutations in our series. Type and distribution of the mutations are given in Table 2. A II carried mutations in 72.7% comparing well with frequencies of 74% [1], 83% [21], 88% [17] and 59% [8] reported in previous studies. *IDH1* mutations in A III were observed in 64.0% comparable to 62% [1], 69% [21], 78% [17] and 52% [8] in earlier studies. The present frequency was for O II 82,0% comparing to 71% [1], 82% [21], 79% [17] and 68% [8] and for O III 69.5% comparing to 67% [1], 86% [21], 75% [17] and 60% [8]. OA II carried *IDH1* mutations in 81.6 % comparing to 78% [1], 100% [21], 94% [17] and 50% [8] and OA III in 66.1% while the previous studies detected mutations in 78% [1], 100% [21],71% [17] and 78% [8]. Thus, the frequencies in the present study are very similar to our previous series but slightly lower than those reported by others. This difference may in part be attributed to small numbers of particular subtypes of gliomas in the other studies, however, the use of different thresholds in scoring weaker signals as sufficient for a mutation may also play a role. Glioma tissues always contain a non-neoplastic cell compartment including vascular cells, reactive astrocytes, lymphocytes and microglial cells. In addition, the diffuse and infiltrative nature of glioma frequently results in a substantial fraction of residual brain in the tissue samples. Therefore, the mutant signal in gliomas heterozygous for mutations usually is less intense than the wild type signal requiring a more or less arbitrary threshold level to separate between a signal indicating a mutation and a background peak.

Mutations in *IDH2* mutations were much less common than those in *IDH1*. We found 31 *IDH2* mutations in our series. A II and A III carried *IDH2* mutation in 0.9% each while 4.7% of O II, 5.3% of O III, 1.3% of OA II and 5.1% of OA III had *IDH2* mutations. Type and distribution of the mutations are given in Table 3. A II carried *IDH2* mutations in 0.9% which is considerably lower than the frequency of 7% previously observed [21]. Mutations in A III were observed in 0.9% and thus were also less frequent than the 4% reported in the preceding study [21]. The present frequency for O II and O III were 4.7% and 5.3% comparing well to 4% and 8% [21]. OA II and OA III carried mutations in 1.3% and 5.1% and have not previously been reported.

IDH1 R132C mutations associate with astrocytoma of WHO grade II

IDH1 mutations exhibited a non-random distribution among astrocytic and oligodendroglial tumors. In our previous study, we hinted at a trend for R132C mutations to favour astrocytomas. In the present series, a total of 29 R132C mutations were observed in 17 A II, 7 A III, 2 O III and 1 OA II and OA III each. This distribution favouring astrocytomas was highly significant ($p < 0,0001$). The significance of the association of R132C with astrocytoma is further supported by a recent report. In a series of Li-Fraumeni patients with astrocytoma, only R132C mutations in *IDH1* but not the much more frequent R132H mutation were observed [18]. Because sporadic astrocytomas carry somatic mutations in the *TP53* gene much more frequently than oligodendrogliomas and oligoastrocytomas, and because Li-Fraumeni patients by definition have a germ line mutation in *TP53*, there seems to be an association of the *IDH1* R132C mutation with mutations in *TP53*. Whether and how *TP53* mutations favour the occurrence of *IDH* R132C mutations, as supported in the Li-Fraumeni setting, or whether *IDH1* mutations favour subsequent *TP53* mutations, as suggested by the significantly higher incidence of *IDH1* mutations than the one of *TP53* mutations in sporadic astrocytomas, remains yet unresolved. R132C mutations were more frequent in WHO grade II tumors than in WHO grade III tumors ($p < 0.05$).

IDH2 mutations associate with oligodendroglial tumors and with the WHO grade III

In our series, *IDH2* mutations predominantly occurred in tumors with an oligodendroglial component. Six O II, 9 O III, 1 OA II and 9 OA III but only 2 A II and A III each carried *IDH2* mutations ($p < 0.001$). This partially contrasts the initial report of *IDH2* mutations in gliomas [21] reporting 2/51 O II, 3/36 O III, but also 2/30 A II and 2/52 A III with *IDH2* mutations. OA II and OA III were not analyzed for *IDH2* in that study. Our findings are very similar with regard to O II and O III, however, they are different from those reported for A II and A III. Divergent results may to some extent be explained by the considerable interobserver variability in the distinction of astrocytoma from oligoastrocytoma [3]. The WHO criteria do allow for a significant diagnostic overlap [12] and the problem is further aggravated by tissue sampling with usually only parts of the tumor tissue being available for histological analysis. Another reason for this discrepancy may be our threshold in scoring mutations resulting in elimination of all R172G mutations reported previously.

In our series 22 of 33 *IDH2* mutations occurred either in A III, O III or OA III. This represents an association with anaplastic tumors ($p < 0.05$). While there is no doubt that *IDH1* mutations occur early

in tumor formation, because the majority of A II, O II and OA II already harbour this alteration, the time point of the occurrence of *IDH2* mutations cannot definitely be set at a similar early point. In fact, the data are also compatible with *IDH2* mutations being associated with tumor progression.

IDH1 and IDH2 are inversely associated

In the initial report on *IDH2* mutations, only glioma samples not carrying *IDH1* mutations were analyzed for *IDH2* [21]. In order to analyze the potentially complementary effects of these mutations, we examined our entire series for both *IDH1* and *IDH2* mutations. We detected 712 tumors with an *IDH1* mutation but wild type for *IDH2*, 27 tumors with *IDH2* mutation but wild type for *IDH1* and only 4 tumors, 1 A III, 1 O III and 2 OA III, characterized by both, *IDH1* and *IDH2* mutations. Interestingly the 4 tumors with mutations in both *IDH1* and *IDH2* were all anaplastic gliomas. This clearly is a non random distribution ($p < 0.00001$). Such partition indicates that either *IDH1* or *IDH2* mutations independently provide a growth advantage for mutant cells and that one of them is sufficient to mediate this advantage. The presence of *IDH2* mutations predominately in anaplastic tumors may indicate that these mutations constitute a progression-associated alteration which promotes more aggressive tumor growth. However, in this case more than only 4 tumors with both, *IDH1* and *IDH2* mutations would have been expected in our series if these mutations arose independent from each other.

IDH1 and IDH2 mutations and age

In patients under the age of 18 years, *IDH1* mutations were rare occurring in only 4 of 32 (12.5%) tumors and *IDH2* mutations were absent. This finding suggests that paediatric astrocytomas, oligodendrogliomas and oligoastrocytomas genetically differ from their adult counterparts. The mean age of adult patients aged 18 or older with anaplastic gliomas of WHO grades III carrying *IDH1* mutations was 43.9 years while anaplastic glioma patients without mutations averaged 50.6 years ($p < 0.0001$). In all three groups, A III, O III and OA III patients with mutations on average were younger than patients without mutations (A III: $p < 0.01$, O III: not significant, OAIII: $p < 0.01$). The average age of patients with gliomas of WHO grade II carrying *IDH1* mutations was 41.3 years while glioma patients without mutations averaged 42.8 years (not significant). In all three groups, A II, O II and OA II patients with mutations on average were older than patients without mutations (not significant). Patients carrying the most common R132H mutation averaged 42.9 years while patients with the

R132C mutation were significantly younger averaging 34.9 years ($p < 0.01$), those with the R132G mutation were 37.9 years old (not significant) and those carrying R132S averaged 36.2 years ($p < 0.01$).

Conclusions

The present study provides a reliable basis for the frequencies and types of *IDH1* codon 132 and *IDH2* codon 172 mutations in diffusely infiltrating astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III. The data confirm a high frequency of *IDH1* mutation in these tumors, with 70.9 % of all investigated tumors carrying an *IDH1* codon 132 mutation, most commonly of the R132H type. In contrast, *IDH2* mutations were restricted to 3.1% of the tumors. Our data confirm a mutually exclusive presence of either *IDH1* or *IDH2* mutation in gliomas. Furthermore, we show that the R132C *IDH1* mutation is significantly associated with astrocytic histology, while *IDH2* mutations are more common in oligodendroglial tumors as compared to astrocytomas. In addition, *IDH2* mutation was more common in anaplastic gliomas of WHO grade III as compared to WHO grade II. Further studies need to address the clinical impact of the individual *IDH1* and *IDH2* mutations with respect to their potential role as prognostic markers in patients with diffuse gliomas.

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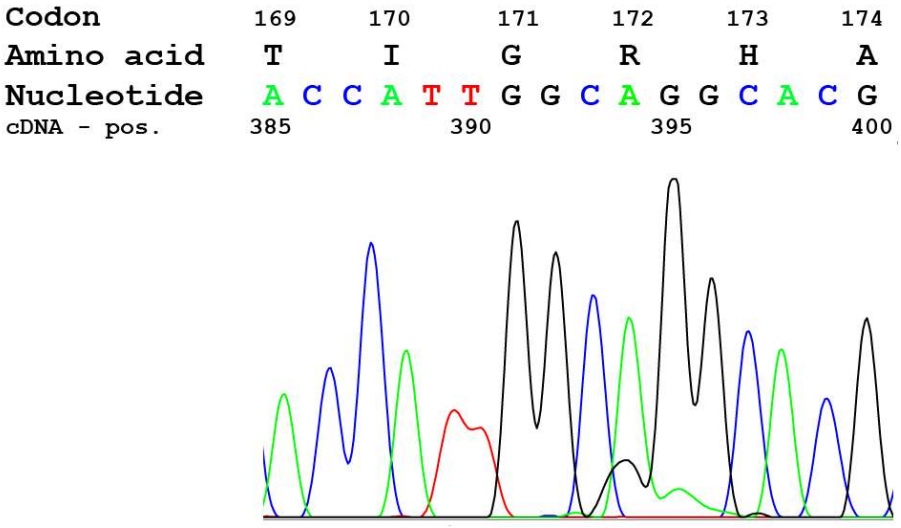
Figure 1



Example for a sequence with a small G – signal at nucleotide position 394 of *IDH2*, which we did not score as evidence for a point mutation but rather considered as a sequencing artefact due to stuttering of polymerase induced by the flanking G signals

Table 1

Type of 716 *IDH1* and 31 *IDH2* mutations and frequency among mutations in 1010 WHO grade II and III astrocytomas, oligodendrogliomas and oligoastrocytomas

Gene	Nucleotide change	Amino acid change	N (%)
IDH1	G395A	R132H	664 (92.7%)
	C394T	R132C	29 (4.2%)
	C394A	R132S	11 (1.5%)
	C394G	R132G	10 (1.4%)
	G395T	R132L	2 (0.2%)
IDH2	G515A	R172K	20 (64.5%)
	G515T	R172M	6 (19.3%)
	A514T	R172W	5 (16.2%)

N (%) = number of tumors and percentage of mutation among all mutations

Table 2

716 *IDH1* codon 132 mutations in 1010 WHO grade II and III astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III

Amino acid exchange	**All tumors N = 1010**	**A II N = 227**	**A III N = 228**	**O II N = 128**	**O III N = 174**	**OA II N = 76**	**OA III N = 177**
R132H	664 (61.7%)	143 (63.0%)	132 (57.9%)	103 (80.5%)	111 (63.8%)	60 (78.9%)	115 (65.0%)
R132C	29 (2.9%)	17 (7.5%)	7 (3.1%)	0 (0%)	2 (1.1%)	1 (1.3%)	1 (0.6%)
R132S	11 (1.1%)	3 (1.3%)	3 (1.3%)	2 (1.5%)	4 (2.3%)	0	0
R132G	10 (1.0%)	2 (0.9%)	3 (1.3%)	0	3 (1.7%)	1 (1.3%)	1 (0.6%)
R132L	2 (0.2%)	0	1 (0.4%)	0	1 (0.6%)	0	0
all	**716 (70.9%)**	**165 (72.7%)**	**146 (64.0%)**	**105 (82.0%)**	**121 (69.5%)**	**62 (81.6%)**	**117 (66.1%)**

N number of tumors analyzed

Table 3

31 *IDH2* codon 172 mutations in 1010 WHO grade II and III astrocytomas, oligodendrogliomas and oligoastrocytomas of WHO grades II and III

Amino acid exchange	**All tumors N = 1010**	**A II N = 227**	**A III N = 228**	**O II N = 128**	**O III N = 174**	**OA II N = 76**	**OA III N = 177**
R172K	20 (2.0%)	2 (0.9%)	2 (0.9%)	3 (2.3%)	6 (3.4%)	1 (1.3%)	6 (3.4%)
R172M	6 (0.6%)	0	0	1 (0.8%)	2 (1.1%)	0	3 (1.7%)
R172W	5 (0.5%)	0	0	2 (1.6%)	1 (0.6%)	0	2 (1.1%)
all	**31 (3.1%)**	**2 (0.9%)**	**2 (0.9%)**	**6 (4.7%)**	**9 (5.3%)**	**1 (1.3%)**	**9 (5.1%)**

N number of tumors analyzed