IDH1 mutation of gliomas with long-term survival analysis

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Abstract. A recurrent mutation affecting codon 132 of the isocitrate dehydrogenase 1 (*IDH1*) gene has been found in ~5% of primary glioblastomas (GBMs), but in >70% of secondary GBMs or oligodendroglial and astrocytic tumors. We investigated *IDH1* mutations in a series of 134 brain tumors to determine the prevalence and prognostic impact of *IDH1* mutations. We also examined the correlations among histology, p53 and PTEN immunoexpression, *MGMT* methylation status, 1p 19q co-deletion and *EGFR* gene amplification. The 134 brain tumors included 41 low-grade oligodendrogliomas (LOs), 47 anaplastic oligodendrogliomas (AOs) and 46 primary GBMs. Data showed that 53.7% (72/134) of cases showed mutations affecting codon 132 of IDH1, including 73.2% of LOs, 82.9% of AOs and three primary GBMs (6.5%). All *IDH1* mutations were Arg132His. In a survival analysis, patients with *IDH1* mutations had better survival compared to those with wild-type *IDH1* ($p<0.05$) in LOs and AOs, but not in primary GBMs ($p=0.587$). In addition, in patients with both IDH1 mutation and MGMT methylation, p53 overexpression was a significant poor prognostic factor both in LOs and AOs. However, IDH1 mutation was not correlated with common genetic profiles that affect patient prognosis, including *MGMT* methylation, 1p 19q co-deletion, PTEN loss and *EGFR* amplification in LOs, AOs and GBMs. From our results, *IDH1* mutation was an independent positive prognostic factor in LOs and AOs, especially in the absence of p53 overexpression.

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Abbreviations: EGFR, epidermal growth factor receptor; FISH, fluorescence *in situ* hybridization; *MGMT*-MSP, O6-methylguanine-DNA methyltransferase methylation-specific polymerase chain reaction

Key words: isocitrate dehydrogenase, mutation, brain tumor, glioma

Introduction

In 2008, genome-wide mutational analysis with 22 glioblastomas (GBM) performed by Parson *et al* (1) found recurrent point mutations affecting the isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* genes. This novel point mutation was thus placed in the spotlight of brain cancer biology (2-29). The mutation in *IDH1* consistently occurred in exon 4 at codon 132, where a CGT→CAT transition of a single amino acid from arginine to histidine (R132H) occurred (1,16), and less frequently in *IDH2*, at the corresponding amino acid R172 (28). *IDH1* and *IDH2* mutations have been found with high frequency in lower grade astrocytic and oligodendroglial neoplasms compared with in GBM (1,2,18,23,28). In addition, although a low incidence of *IDH1* mutation was found in primary GBM, it is more frequently mutated in secondary GBM (2,28). The accumulating research regarding this *IDH1* mutation has generated many insights (30-33); one is the prognostic usefulness of *IDH1* mutation, as mutated tumors have a better prognosis. The other is that *IDH1* mutation has already become an essential diagnostic marker for brain tumors (1,22,28,34). Also, in a multivariate analysis, *IDH1* mutation was confirmed as an independent prognostic factor in patients with gliomas (18,23). Among the notable genetic profiles in gliomas, 1p 19q co-deleted genotype and *MGMT* methylation were tightly associated with *IDH1* mutation, but *IDH1* mutation was mutually exclusive with *EGFR* gene amplification and loss of chromosome 10 (23). Substantial research effort into *IDH1* mutation has concentrated on its mechanistic role. Wild-type *IDH1* catalyzed the oxidative carboxylation of isocitrate (ICT) to α-ketoglutarate (α-KG), yielding reduced nicotinamide adenine dinucleotide phosphate (NADPH) (23). However, mutated *IDH1* in a tumor inhibited *IDH1*-mediated conversion of ICT to α-KG and induced hypoxia-inducible factor 1α (HIF-1α) (29). Moreover, mutated *IDH1* acquired the ability to catalyze α-KG to R(-)-2-hydroxyglutarate [R(-)-2HG] (6,20). Identification of *IDH1* mutation in gliomas not only improved physicians' ability to predict disease progression but also prompted researchers to re-evaluate the disease entity. The study of *IDH1* mutations will change the treatment options and drug regimens used in gliomas in the near future. Although the need for such research is increasing, no information is available regarding *IDH1*/IDH2 mutations in Korean brain tumor patients. Therefore, to determine the prevalence and prognostic impact of *IDH1*/*IDH2* mutations in the Korean population,

we investigated a series of 134 glioma patients. Additionally, we compared *IDH1* mutations with other genomic profiles commonly associated with gliomas.

Materials and methods

Case selection. Tumor tissue was from human brain tumor specimens diagnosed in the Department of Neuropathology at the Seoul National University Hospital from 1999 to 2011. This study included 41 oligodendrogliomas (LO), 47 anaplastic oligodendrogliomas (AO), and 46 primary GBM. This study was approved by the Institutional Review Board of Seoul National University Hospital (H-1201-037-394).

DNA extraction and PCR amplification for IDH1 sequencing. Tumor areas were manually microdissected from 6-μm unstained histological sections. DNA was isolated from tumor tissue using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Template DNA (1 μl) was added to 100 μl of PCR reaction solution [10 μl 10X MG™ Taq-*HF* buffer, 10 μl 2 mM MG™ dNTP mixture, 5 μl 10 pmol primer (2X), 1 μl MGTaq-*HF* polymerase, distilled water]. IDH1 forward primer (5'-ACC AAA TGG CAC CAT ACG A-3') and reverse primer (5'-GCA AAA TCA CAT TAT TGC CAA C-3') generated a 130-bp PCR product; IDH2 forward primer (5'-GCT GCA GTG GGA CCA CTA TT-3') and reverse primer (5'-TGT GGC CTT GTA CTG CAG AG-3') generated a 293-bp PCR product (Table I). PCR amplification was performed using AmpliTaq Gold PCR Master Mix (Applied Biosystems, Inc., Foster City, CA). The reaction mixture was subjected to an initial denaturation at 95˚C for 10 min, followed by 35 cycles of amplification consisting of denaturation at 95˚C for 30 sec, annealing at 55˚C for 30 sec, and extension at 72˚C for 60 sec.

Direct sequencing. Purified PCR products were sequenced using two IDH1 primers, as described in Table I. Sequencing was performed using a BigDye terminator cycle sequencing kit v.3.1. (Applied Biosystems). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems).

Immunohistochemistry. The primary antibodies used in the immunohistochemical study of formalin-fixed, paraffin-embedded sections are summarized in Table II, based on our previous reports (35,36).

Fluorescence in situ hybridization (FISH). Analysis of chromosome 1p, 19q deletion, and *EGFR* gene status was conducted by FISH using Vysis probes, as per our previous report (35,36).

O6-methylguanine-DNA methyltransferase (MGMT) methylation-specific polymerase chain reaction (MSP) analysis. Analysis of methylation of the *MGMT* promoter was performed using the methylation-specific polymerase chain reaction (MSP) technique, as described previously (37).

Statistical analyses. Fisher's exact test was used to examine associations between *IDH1* mutations and genetic alterations. The t-test was used to assess the relationship of *IDH* mutations with the absence or presence of genetic alterations with age. Overall survival of patients with LO, AO was estimated by the Kaplan-Meier method and compared using a log-rank test. All statistical analyses were performed with SPSS version 18 (SPSS Inc., Chicago, IL, USA).

Table I. Amplification and sequencing primers.

Name	Sequence
IDH1-F	5'-ACC AAA TGG CAC CAT ACG A-3'
IDH1-R	5'-GCA AAA TCA CAT TAT TGC CAA C-3'
IDH2-F	5'-GCT GCA GTG GGA CCA CTA TT-3'
IDH2-R	5'-TGT GGC CTT GTA CTG CAG AG-3'

IDH, isocitrate dehydrogenase; F, forward; R, reverse.

Table II. Antibodies used in this study.

Name	Manufacturer	Antigen retrieval	Dilution
P53	Dako, Glostrup, Denmark	EDTA, microwave	1:800
PTEN	Dako, Glostrup, Denmark	EDTA, microwave	1:100

Results

IDH1 mutation frequencies in various brain tumors. The patients ranged in age from 3 to 71 years (mean 41.3 years). The male-to-female ratio was 1.5:1. We analyzed DNA from 134 formalin-fixed, paraffin-embedded tissue samples from archival surgical specimens. We found 72 (53.7%) mutations in codon 132 of IDH1 (Fig. 1). All were G395A (Arg132His). *IDH1* mutation frequencies differed according to histologic subtype, affecting 30 (73.2%) of 41 LO, 39 (82.9%) of 47 AO and three (6.5%) of 46 primary GBM cases at *IDH1* codon 132. These results are summarized in Table III.

Oligodendroglioma (LO). The 41 LO patients ranged in age from 23 to 69 years (mean 41.1 years). They were operated on from 1999 to 2009. The follow-up duration was 9.9 to 130.5 months. Tumor recurrence occurred in 14 cases, and one case was lost to follow-up. Of the 14 patients with recurrence, 12 had an *IDH1* mutation. Of the 26 patients without recurrence, 18 had an *IDH1* mutation. Of the 38 patients whose samples were subjected to p53 immunostaining, 8 revealed simultaneous p53 expression and *IDH1* mutation. Sixteen of 20 patients whose samples were subjected to PTEN immunostaining expressed PTEN and the *IDH1* mutation. The 1p 19q co-deletion was found in 32 of 38 patients by FISH. Of the 32 patients with the 1p 19q co-deletion, 22 had the *IDH1* mutation. None of our LO revealed EGFR gene amplification or high polysomy. In LO, we analyzed the relationship between the *IDH1* mutation and factors, such as age, sex, recurrence and 1p 19q co-deletion. There was no statistically

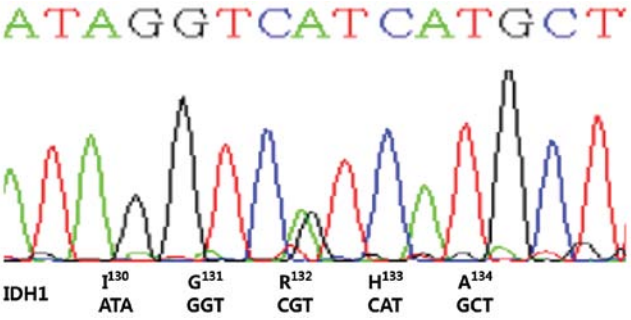

Figure 1. IDH1 mutation study was carried out in formalin-fixed paraffin embedded tissue. All of IDH1 mutated cases had CGT→CAT transition change, a single amino acid from arginine to histidine (R132H).

Table III. *IDH1* mutation frequencies in 134 brain tumors.

	IDH1			
	Mutant	Wild	Total number	Percent (%)
LO	30	11	41	73.2
AO	39	8	47	82.9
Primary GBM	3	43	46	6.5
Total	72	62	134	53.7

LO, low grade oligodendrogliomas; AO, anaplastic oligodendrogliomas; GBM, glioblastoma.

significant correlation between *IDH1* mutation status and other factors except p53. In patients with both *IDH1* mutation and *MGMT* methylation, p53 immunoexpression was a significant negative prognostic factor (p=0.049) (Fig. 3).

Anaplastic oligodendroglioma (AO). The 47 AO patients ranged in age from 26 to 69 years (mean 44.9 years). Of the 12 patients with recurrence, 10 had the *IDH1* mutation. Of the 35 patients without recurrence, 29 had the *IDH1* mutation. Of the 42 patients whose samples were subjected to p53 immunostaining, 6 revealed simultaneous p53 expression and *IDH1* mutation. Twenty of 37 patients whose samples were subjected to PTEN immunostaining expressed PTEN and the *IDH1* mutation. The 1p 19q co-deletion was found in 40 of 47 patients by FISH. Of the 40 patients with the 1p 19q co-deletion, 33 had the *IDH1* mutation. Seven cases without the 1p 19q co-deletion had the *IDH1* mutation. *EGFR* FISH was performed in 47 cases. Forty-five of these revealed no amplification of the *EGFR* gene, and 37 of these had the *IDH1* mutation. Only 2 cases of AO showed *EGFR* FISH-positive (2 high polysomy). These two *EGFR* FISH-positive cases had the *IDH1* mutation. Thus, we could not see the mutual exclusion between IDH1 mutation and EGFR positivity. Forty-one of 43 cases subjected to *MGMT* MSP revealed methylation of the *MGMT* promoter, and 35 of these had the *IDH1* mutation. In AO, there was no statistically significant correlation between the *IDH1* mutation and other factors, such as sex, recurrence, 1p 19q co-deletion, *EGFR* FISH. In the patients with both IDH1 mutation and MGMT methylation, p53 immunoexpression was a significant negative prognostic factor as in LO (p=0.002) (Fig. 3).

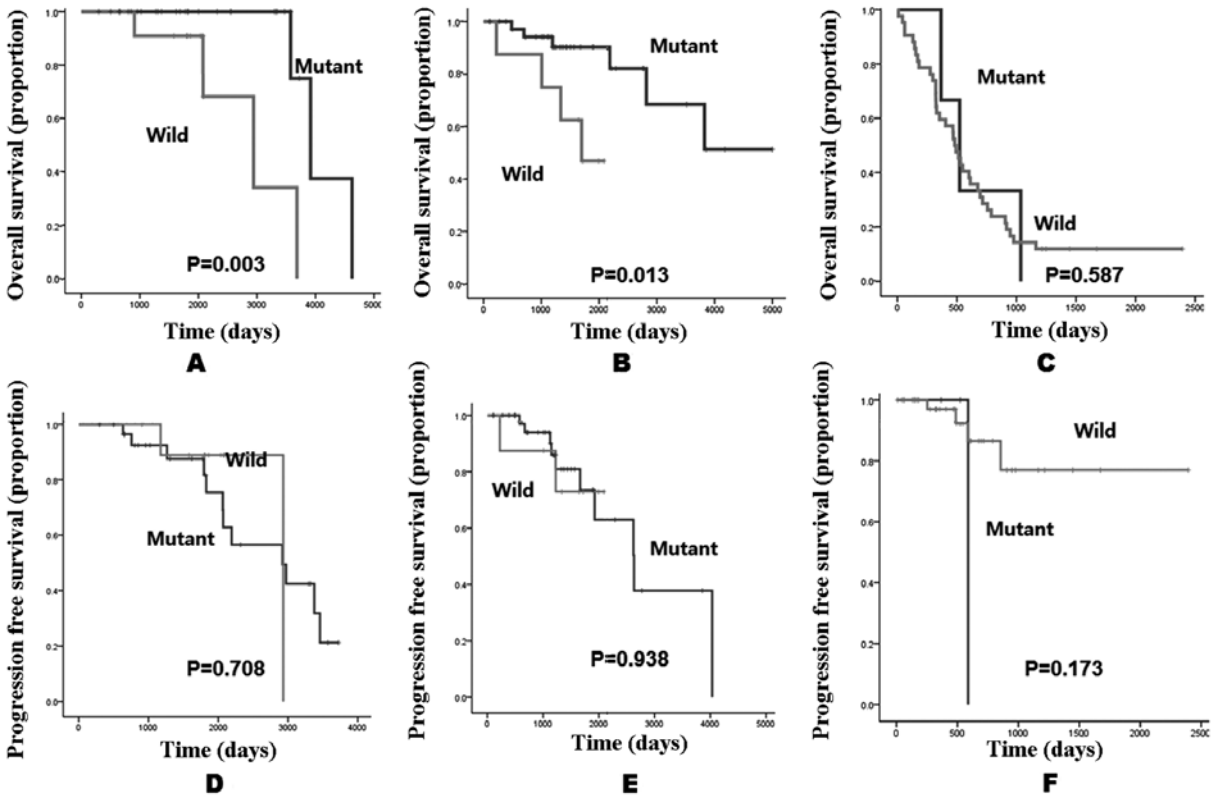

Figure 2. Overall survival (OS) and progression-free survival (PFS) curves of IDH1 mutation according to histologic subgroup. OS curve in LO (A), AO (B), and GBM (C). PFS curve in LO (D), AO (E) and GBM (F).

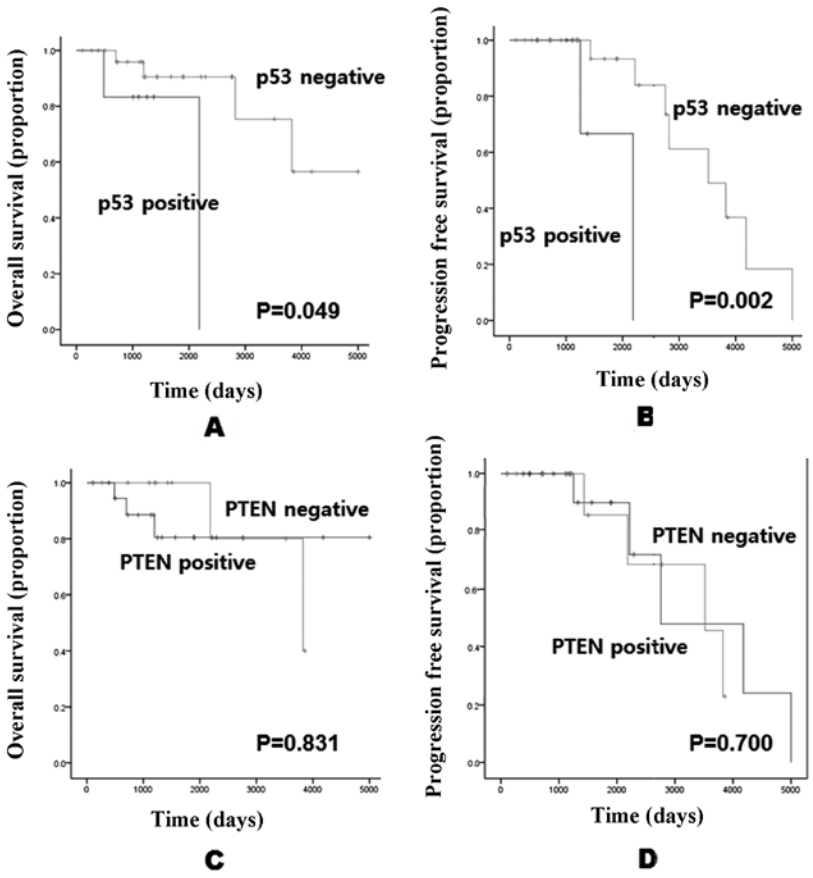

Figure 3. (A) Overall survival and (B) progression-free survival curves of IDH1 mutated and MGMT-methylated AO according to p53 expression. (C) Overall survival and (D) progression-free survival curves of IDH1 mutated and MGMT-methylated AO according to PTEN expression.

Glioblastoma (GBM). The 46 GBM patients ranged in age from 3 to 71 years (mean 39.6 years). Among the 41 patients without recurrence, two had the *IDH1* mutation, and 39 did not. Of the 5 patients with recurrence, one had the *IDH1* mutation. The 1p 19q co-deletion was not found in any of the 46 patients by FISH. Three of 46 cases without the 1p 19q co-deletion had the *IDH1* mutation. *EGFR* FISH was positive in 7 (15.2%) out of 46 performed. One of 7 cases with *EGFR* gene amplification and 2 of 39 patients without EGFR gene amplification had the IDH1 mutation. Therefore, EGFR gene amplification and IDH1 mutation were not mutually exclusive (p=0.398), but it needs to be studied in more cases. Fourteen (30.4%) cases of 46 GBM performed *MGMT* MSP revealed methylation of the *MGMT* promoter. Two of 14 cases with MGMT methylation and one of 32 cases without methylation of the *MGMT* promoter had the *IDH1* mutation. We analyzed the relationship between *IDH1* mutation status and other factors, such as sex, recurrence, 1p 19q co-deletion, *EGFR* FISH and *MGMT* MSP and found no statistically significant correlation.

Survival analysis. We analyzed the prognostic impact of *IDH1* mutation in LO, AO, and GBM. The follow-up duration was 9.93-130.5 months, 3.4-166.6 months and 9 days to 79.8 months in LO, AO and GBM. The median survival was 68.4, 54.2 and 19.7 months in LO, AO and GBM, respectively. The overall survival rate was 82.9, 78.7 and 0% in LO, AO and GBM, respectively. In GBM, 1-, 2- and 3-year survival rates were 60.9, 28.4 and 13.0%, respectively. In LO, overall survival was higher in *IDH1*-mutated LO, compared with non-mutated LO (p=0.03; Fig. 2A). Also, in AO, overall survival was higher in *IDH1* mutated AO compared with non-mutated AO (p=0.013; Fig. 2B). In contrast to LO and AO, overall survival was higher in *IDH1* non-mutated GBM, compared with mutated GBM (p=0.587; Fig. 2C), but this result was not statistically significant, because *IDH1* mutated GBM was only 3 cases. Also, progression-free

Table IV. The relationship of *IDH1* mutations to genetic alterations according to age.

Entity	IDH1	Number	Mean age	SD	P-value
LO	Mutant	30	41.67	10.1	0.578
	Wild	11	39.55	12.34	
AO	Mutant	39	45.77	10.22	0.856
	Wild	8	45.5	11	
GB	Mutant	3	48.67	10.26	0.456
	Wild	43	39.37	21.04	

SD, standard deviation; LO, low grade oligodendrogliomas; AO, anaplastic oligodendrogliomas; GBM, glioblastoma.

survival was not statistically different between *IDH1* mutated and non-mutated tumors in the LO (p=0.708; Fig. 2D), AO (p=0.938; Fig. 2E), and GBM (p=0.173; Fig. 2F) groups. We analyzed survival in patients with both *IDH1*-mutated and MGMT methylated LO and AO according to p53 and PTEN expression. The overall survival and progression-free survival of patients with p53-negative tumors were significantly longer than in those with p53-positive tumors (p=0.049 and 0.002; Fig. 3A and B). However, PTEN expression did not affect the patients' survival.

Discussion

Recently, *IDH1* mutation has been recognized as a strong prognostic factor in brain tumors. In low-grade astrocytic and oligodendroglial tumors (WHO grades II and III), the impact on prognosis was magnified several times. However, no Korean studies evaluating *IDH1* mutation frequencies in brain tumors had previously been conducted, so we attempted to discover the *IDH1* mutation frequency and prognostic impact in a Korean patient population. The methods for detection of *IDH1* mutation have changed. Recently, a monoclonal antibody that detects the R132H *IDH1* mutation was developed. This antibody has been applied to everyday pathologic practice (5). Immunohistochemical research with this monoclonal antibody is easy, and inexpensive, but has some limitations. The best way to identify the *IDH1* mutation is by direct sequencing; thus, we performed direct sequencing instead of immunohistochemistry for *IDH1*. According to our data, WHO grades II and III astrocytic and oligodendroglial tumors exhibit high mutation frequencies. Although the case numbers were insufficient to reflect tumor frequencies, our *IDH1* mutation results were similar to other published results. In accordance with other studies, we found that *IDH1* mutation was a strong prognostic factor in oligodendroglioma and anaplastic oligodendroglioma. Detection of the *IDH1* mutation was extremely helpful in brain tumors. Using the same approach with the 1p 19q co-deletion, IDH 1 mutation was an excellent prognostic marker. The usefulness of the *IDH1* mutation in brain tumors has been reported previously. In addition to its predictive utility, *IDH1* also has possibilities as a diagnostic marker. *IDH1* is a powerful differential diagnostic marker for round and clear-cell brain tumors mimicking oligodendroglioma, including dysembryoplastic neuroepithelial tumor (DNT), extraventricular neurocytoma (EVN), and clear-cell ependymoma, because the *IDH1* mutation is not present in these or other glioneuronal tumors, such as central neurocytoma and gangliogliomas. Therefore, we performed additional sequencing to detect the *IDH1* mutation in 5 cases of PGNT and 11 of EVN. The *IDH1* mutation was not detected in these tumor entities, however, the number of cases was small. Thus, we suggest the *IDH1* mutation as a reasonable adjunctive analysis in daily practice, especially for diagnosis of brain tumors with clear-cell morphology.

We analyzed the relationship between *IDH1* mutation and other aspects of the genetic profile, including 1p 19q co-deletion, EGFR amplification, and MGMT methylation in LO, AO, and GBM. In LO and AO, the *IDH1* mutation was more frequent in tumors with the 1p and 19q co-deletion than those without 1p 19q co-deletion. Also, in AO, the *IDH1* mutation was more frequent in MGMT-methylated tumors. Although none of these findings was statistically significant, these data are similar to those in other reports. The statistical significance of these associations between genetic profile and the *IDH1* mutation should be verified in a larger series. In contrast to the report by Hartmann *et al* (13), the mean age of the two groups in this study was not significantly different. These results are summarized in Table IV.

In conclusion, we identified *IDH1* mutation frequencies in various brain tumors; *IDH1* mutation was a strong prognostic factor in gliomas. Besides its role as a prognostic marker, the *IDH1* mutation may be a useful diagnostic marker. We also confirmed the lack of a difference in mean age between the *IDH1* wild-type and mutant groups. Among patients with both *IDH1* mutation and MGMT methylation, p53 immunoexpression was a significant negative prognostic factor in both LO and AO, but PTEN loss did not affect these patients' survival. However, we found no association between *IDH1* mutation and other genetic profiles commonly associated with gliomas. These results remain to be proven in a larger study.

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