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ORIGINAL ARTICLE

Clinical, histopathological, and molecular analyses of *IDH*-wild-type WHO grade II–III gliomas to establish genetic predictors of poor prognosis

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Abstract
The genetic features of *isocitrate dehydrogenase*-wild-type (*IDH*-wt) lower-grade gliomas (LGGs; World Health Organization grades II and III) are not well defined. This study analyzed the genetic and other features of *IDH*-wt LGGs to develop a subclassification that can be used to predict their prognosis. Clinical, histopathological, and genetic features of 35 cases of diffuse *IDH*-wt astrocytoma and *IDH*-wt anaplastic astrocytoma were analyzed. The following genetic factors were examined: mutations of *B-rapidly accelerated fibrosarcoma*, *telomerase reverse transcriptase* promoter (*TERT*p), *histone 3 family 3A*, and *alpha-thalassemia/mental retardation syndrome*, *X-linked*; and copy number aberrations. In the univariate analysis, the following factors were associated with poor overall survival (OS): the histopathological diagnosis, *TERT*p mutation, the gain of chromosome 7 (+7), and the loss of chromosome 10q (−10q). In the multivariate analysis, +7, −10q, and *TERT*p mutation were independent prognostic factors associated with poor OS. The median OS was significantly worse for patients who harbored at least one of these factors than for those without any of them (18.5 vs. 54.5 months, $P=0.002$). The subclassification of *IDH*-wt LGGs according to the genetic factors + 7, − 10q, and *TERT*p mutation is potentially useful for predicting the prognosis.

Keywords Chromosome 7 · Chromosome 10 · Glioma · *IDH*-wild-type · *TERT* promoter

Introduction

The 2016 World Health Organization (WHO) Classification of Tumours of the Central Nervous System classifies *isocitrate dehydrogenase* (*IDH*)-mutant lower-grade gliomas (LGGs) according to the status of the *IDH1* and *IDH2* genes, further subdividing them by molecular characteristics into astrocytic or oligodendroglial tumors. In contrast, genetic characteristics to subclassify *IDH*-wild-type (*IDH*-wt) LGGs have not yet been elucidated [1].

Several factors have been reported to be associated with the overall survival (OS) of patients with *IDH*-wt LGGs. These include age, the extent of resection, WHO grade, and the following genetic alterations: the K27M mutation of *histone 3 family 3A* (*H3F3A*; H3-K27M); the V600E mutation of *B-rapidly accelerated fibrosarcoma* (*BRAF*); mutation of *human telomerase reverse transcriptase* promoter (*TERT*p); amplification of *epidermal growth factor receptor* (*EGFR*); and combinations of the total or partial gain of chromosome 7 (+7) and the total or partial loss of chromosome 10 (−10) [2–8].

Some of these genetic alterations have also been detected in glioblastomas; indeed, it has been reported that the clinical course of *IDH*-wt LGGs with these alterations is similar to that of *IDH*-wt glioblastoma [3, 5].

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It is thought that *IDH*-wt glioblastomas may sometimes be misdiagnosed as *IDH*-wt LGGs, especially with small specimens and those that lack the histopathological features of glioblastoma, such as microvascular proliferation or palisading necrosis [4].

Hirose et al. subclassified *IDH*-wt LGGs according to their DNA copy number aberrations, particularly of chromosomes 7 and 10 [9, 10]. The alteration + 7 has frequently been detected in astrocytic tumors [10], and the loss of chromosome 10q (− 10q) has been detected in anaplastic astrocytoma (AA) and glioblastoma [10, 11]. The combination of genetic alterations + 7 and − 10q is commonly found in *IDH*-wt glioblastomas [4–6, 8].

In normal cells, each cell division shortens the length of the telomeres, whereas telomeres are often lengthened by telomerase in cancer cells [12]. Telomerase comprises an RNA subunit and a telomerase reverse transcriptase catalytic subunit; it maintains or increases the length of the telomere by adding telomeric repeats to the ends of the chromosome [12]. *TERT*p plays an important role in telomerase activation through transcriptional regulation. C228T or C250T are typical hot spots of *TERT*p mutation and are often detected in *IDH*-wt glioblastomas and oligodendrogliomas [5, 8]. Another mechanism that maintains telomere length is known as alternative lengthening of telomeres. Mutation of the *alpha-thalassemia/mental retardation syndrome*, *X-linked* (*ATRX*) has been detected in 7% of primary glioblastomas and has been shown to be associated with alternative lengthening of telomeres development [13]. It has been reported that *ATRX* and *TERT*p mutations are mutually exclusive and that *TERT*p mutation but not *ATRX* mutation is associated with a poor prognosis in *IDH*-wt LGGs [2, 3, 5–8].

H3-K27M is frequently detected in gliomas of the brainstem, thalamus, and spinal cord [1]. H3-K27M-mutant pediatric glioma located in midline structure is diagnosed diffuse midline glioma, H3-K27M mutant and is classified as WHO grade IV [1]. H3-K27M has been reported to be an unfavorable prognostic factor for supratentorial *IDH*-wt LGGs [2, 3].

Although the *BRAF* V600E mutation has been detected less frequently in glioblastomas, it has been proposed as a potential indicator of better prognosis [14–16]. This mutation has been detected in 43–66% of pleomorphic xanthoastrocytomas (PXAs), 65% of anaplastic PXAs, 0–3% of adult LGGs, and 2% of glioblastomas, and is considered an important hallmark of PXA and anaplastic PXA [14].

The aim of the present study was to analyze the prognostic value for *IDH*-wt LGGs of copy number alterations of chromosomes 7 and 10 and mutations of *TERT*p, *BRAF*, *H3F3A*, and *ATRX*, and then to use the results of this analysis to develop a subclassification of *IDH*-wt LGGs that can be used to predict their prognosis.

Materials and methods

Patients and tissue samples

This study was approved by the Ethics Committee of Fujita Health University (Approval number: GH18-005). Our study included 41 Japanese patients with adult supratentorial *IDH*-wt LGG, primarily resected at Fujita Health University Hospital between 2004 and 2018. The histopathological diagnoses included PXA, anaplastic PXA, diffuse astrocytoma (DA), and AA. The status of the *IDH1* and *IDH2* was confirmed by Sanger sequencing, and the patients' tissue samples were rediagnosed according to 2016 WHO classification.

Clinical data

Clinical information was obtained retrospectively from the patients' electronic medical records. Age (< 50 years or ≥ 50 years) and Karnofsky Performance Scale score (< 80 or ≥ 80) at the time of diagnosis were recorded. All the patients underwent enhanced magnetic resonance imaging (MRI) before surgery. Calcification was evaluated histopathologically. The extent of tumor removal was classified as gross total resection (defined as macroscopic complete removal of ≥ 95% of the initial tumor volume), subtotal resection (removal of 50–95% of the initial tumor volume), or partial resection or biopsy (removal of < 50% of the initial tumor volume) [17, 18]. The extent of tumor resection was evaluated by comparing the preoperative and postoperative MR imaging [17–19].

DNA preparation

The tumor samples were available as frozen tissues and/or as formalin-fixed paraffin-embedded (FFPE) samples. DNA was extracted from the frozen tissue samples using DNeasy Blood & Tissue Kits (QIAGEN, Hilden, Germany) and from the FFPE samples using DNA FFPE Tissue Kits (QIAGEN), according to the manufacturer's instructions. The quality of the DNA was assessed by absorption analyses.

Comparative genomic hybridization

Comparative genomic hybridization was performed as previously described [9]. According to pathological appearance or MIB-1 density, tumor tissues were obtained from FFPE samples and the tumor DNA was amplified by degenerate oligonucleotide-primed polymerase chain reaction (PCR). Control DNA was obtained from the blood lymphocytes of healthy donors. After amplification, the DNA from the

samples was labeled with biotin-labeled deoxyuridine triphosphate (Roche, Basel, Switzerland), and the labeled DNA was hybridized with that from normal tissues to create normal metaphase spreads. After washing away any unhybridized probes, the spreads were counterstained with 4,6-diamino-2-phenylindole and the fluorescence intensity ratio of each chromosome was evaluated with CytoVision software (Applied Imaging, San Jose, CA, USA).

Mutational analysis

Mutational analyses of *IDH1/2*, *BRAF*, *TERT*p, and *H3F3A* were performed using PCR and Sanger sequencing, as previously described [20–24]. The following sequences were analyzed: codon 132 for *IDH1*, codon 172 for *IDH2*, codon 600 for *BRAF*, codons 228 and 250 for *TERT*p, and codon 27 for *H3F3A* [20–24]. The primers used are presented in Table 1. The sequence analysis was performed using an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Immunohistochemistry

Immunohistochemistry was performed to measure the MIB-1 index and evaluate *ATRX* mutation, as previously described [25, 26]. The MIB-1 index was evaluated with anti-Ki67 antigens (BioGenex Laboratories, Fremont, CA, USA) and calculated as the percentage of positively stained tumor cell nuclei with anti-Ki67 antibodies in a total of 1000 tumor cell nuclei [25]. The median MIB-1 index for the 35 cases with *IDH*-wt DA or *IDH*-wt AA included in the analysis was 18.2; we, therefore, defined MIB-1 ≥ 18.2 as high and MIB-1 < 18.2 as low. *ATRX* mutation was assessed using anti-*ATRX* antibodies (Merek KGaA, Darmstadt, Germany) [26]. Samples were classified as *ATRX*-positive or *ATRX*-deficient according to whether the neoplastic cells had ≥ 10% or < 10% positive nuclear staining, respectively. Reactive cells and endothelial cells were generally *ATRX*-positive and served as an internal positive control. *ATRX*-deficiency was used as a surrogate for *ATRX* mutation, as described in previous reports [12, 16, 27].

Table 1 The primers used for polymerase chain reaction in our study

Gene symbol	Sequence
IDH1	
Sense	5′-CGGTCTTCAGAGAAGCCATT-3′
Antisense	5′-CACATTATTGCCAACATGAC-3′
IDH2	
Sense	5′-CTCACAGAGTTCAAGCTGAAGAAG-3′
Antisense	5′-CTGTGGCCTTGTACTGCAGAG-3′
*TERT*p	
Sense	5′-CAGCGCTGCCTGAAACTC-3′
Antisense	5′-GTCCTGCCCCTTCACCTT-3′
H3 F3A	
Sense	5′-AATTTCCAGATTTGGGGAGG-3′
Antisense	5′-GCAAAAAGTTTTCCTGTTATCCA-3′
BRAF	
Sense	5′-TGCTTGCTCTGATAGGAAAATG-3′
Antisense	5′-AGCATCTCAGGGCCAAAAAT-3′

BRAF B-rapidly accelerated fibrosarcoma, *H3F3A histone 3 family 3A*, *IDH isocitrate dehydrogenase*, *TERTp telomerase reverse transcriptase* promoter

Statistical analysis

The patients' prognoses were evaluated based on OS, defined as the period from the date of first surgery until the date of death or the last follow-up. Univariate and multivariate analyses were performed using EZR v1.37 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [28]. For the univariate analysis, the Kaplan–Meier method was used to plot survival curves and the log-rank test was applied to evaluate the comparisons between the different groups. Statistical significance was considered to be $P < 0.05$. The factors in the univariate analysis with $P < 0.05$ were included in the multivariate analysis, using a Cox proportional hazards model to evaluate hazard ratios (HRs) with 95% confidence intervals (CIs) for OS for each factor.

Results

Patient characteristics and clinical outcomes

From the initial 41 cases, we excluded six cases with PXA or anaplastic PXA for the main analysis. The clinical data for the remaining 35 patients (20 men and 15 women) are summarized in Fig. 1. The mean age was 59 years (range 23–81 years). Eight cases were of *IDH*-wt DA (WHO grade II) and 27 were of *IDH*-wt AA (WHO grade III). Gross total resection, subtotal resection, and partial resection/biopsy were performed for 14, 8, and 13 cases, respectively. After the surgical procedures, 28 patients underwent chemotherapy (using temozolomide, bevacizumab, and nimustine) and 26 received radiation therapy.

The median OS for all 35 patients was 28.9 months. Patients aged ≥ 50 years at diagnosis tended to have shorter OS compared with those aged < 50 years (median OS, 32.8 vs. 24.6 months, $P = 0.082$). The median OSs for *IDH*-wt AA and *IDH*-wt DA were 24.6 and 41.4 months, respectively ($P = 0.031$; Fig. 2a). Sex, Karnofsky Performance Scale score, enhancement on MRI, extent of resection,

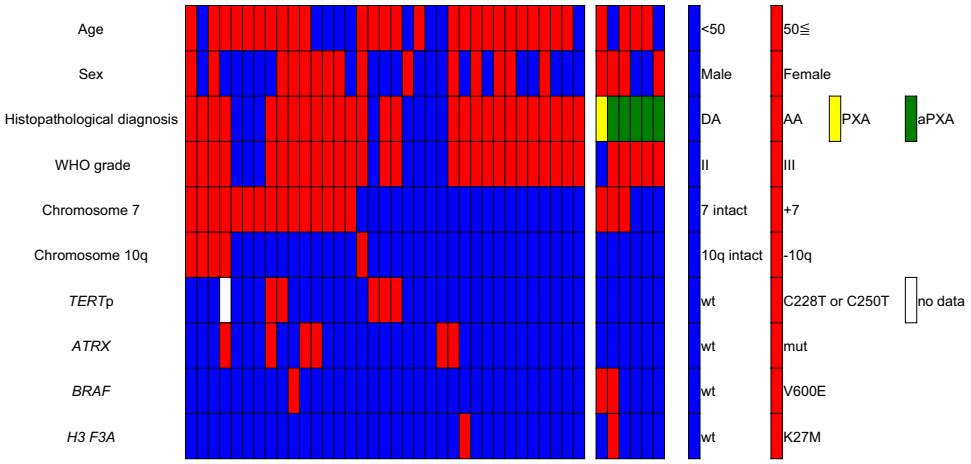

Fig. 1 Clinical, histopathological and genetic characteristics of the 41 *IDH*-wild-type WHO grade II–III gliomas. *AA* anaplastic astrocytoma, *aPXA* anaplastic pleomorphic xanthoastrocytoma, *ATRX alpha-thalassemia/mental retardation syndrome, X-linked*, *BRAF B-rapidly accelerated fibrosarcoma*, *DA* diffuse astrocytoma, *H3F3A histone 3 family 3A*, *mut* mutation, *PXA* pleomorphic xanthoastrocytoma, *TERTp telomerase reverse transcriptase* promoter, *WHO* World Health Organization, *wt* wild-type, + *7* gain of chromosome 7, − *10q* loss of chromosome 10q

histopathological calcification, MIB-1 index, chemotherapy, and radiation therapy showed no significant association with OS. These results are summarized in Table 2.

Copy number aberration

The copy number aberration + 7 was detectable in 15 of the 35 cases (43%). The median OS of these patients was 20.9 months, compared with 41.4 months for patients without + 7 ($P < 0.011$; Fig. 2b). Five cases showed − 10q, with a median OS of 10.3 months compared with 29.7 months for the patients without − 10q ($P = 0.01$; Fig. 2c).

Genetic characteristics

*TERT*p mutation was detected in 5 of 34 cases (15%; data were not available for one case). Three cases showed the C228T mutation and two cases the C250T mutation. One case was of *IDH*-wt DA and four were of *IDH*-wt AA. Patients with *TERT*p mutation tumors had shorter OSs compared with those with *TERT*p wild-type tumors (median OS 15.9 months vs 30.0 months, $P = 0.034$; Fig. 2d). H3-K27M was detected in one case of *IDH*-wt AA, and the *BRAF* V600E mutation in one case of *IDH*-wt DA. *ATRX* mutation was detected in 6 of the 35 cases (17%) but was not significantly associated with OS.

Multivariate analysis

The multivariate analysis of clinical and genetic factors with $P < 0.05$ in the univariate analyses demonstrated the independent prognostic value of three factors: + 7 (HR 5.00, 95% CI 1.47–16.67, $P = 0.01$), − 10q (HR 12.5, 95% CI 2.38–50.00, $P = 0.002$), and *TERT*p mutation (HR 7.69, 95% CI 1.43–50.00, $P = 0.018$) in OS (Table 3).

Subclassification by + 7, − 10q, and *TERT*p mutation

The cases were divided into two groups according to whether or not they harbored any of the three significant prognostic factors: + 7, − 10q, and/or *TERT*p mutation. One or more of these factors were found in 19 of the 35 cases (54%), including four cases of *IDH*-wt DA and 15 cases of *IDH*-wt AA. These patients showed shorter OS compared with the patients with none of the prognostic factors (median OS 18.5 months vs. 54.5 months, $P = 0.002$; Fig. 3a). For the eight cases of *IDH*-wt DA, the median OS of the four with at least one of the prognostic factors was 29.0 months, whereas that of the other four cases was not reached (Fig. 3b). For the 27 cases of *IDH*-wt AA, the median OS for the 15 cases with at least one of the prognostic factors was significantly shorter than that for the cases without any of them (16.6 months vs. 41.7 months, $P = 0.036$; Fig. 3c). These results suggested that subclassification according to these three genetic factors was more useful than WHO grades for predicting the prognosis of patients with *IDH*-wt LGGs. Moreover, we subdivided the 19 cases of the tumor with + 7, − 10q and/or *TERT*p mutation into two groups: tumor with only + 7 and tumor with − 10q and/or *TERT*p mutation. Median OS of patients harboring tumor with only + 7 was 29.7 months, whereas that of patients harboring tumor with − 10q and/or *TERT*p mutation was 15.9 months, which was statistically different ($P = 0.032$).

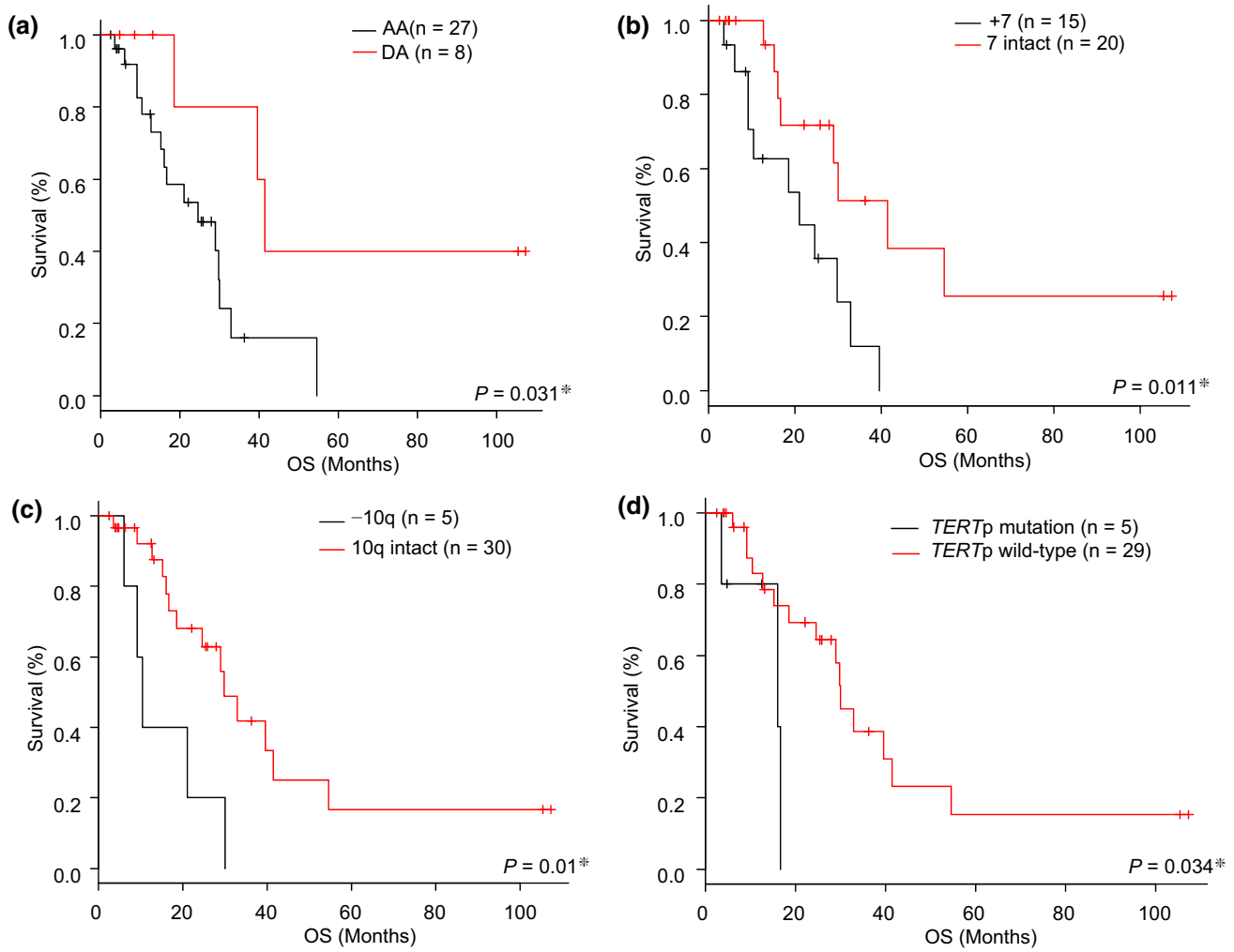

Fig. 2 Kaplan–Meier analysis of OS according to the clinical and molecular factors with *P* values < 0.05 in the univariate analysis. **a** The histopathological diagnosis; **b** + 7; **c** − 10q; and **d** *TERT*p mutation. *AA* anaplastic astrocytoma, *DA* diffuse astrocytoma, *OS* overall survival, *TERTp telomerase reverse transcriptase* promoter, + *7* gain of chromosome 7, − *10q* loss of chromosome 10q. *$P < 0.05$

Analysis including the cases with PXA

Because histopathological diagnoses of PXA can sometimes be confused with astrocytoma, we reanalyzed all 41 cases, including those with PXA and anaplastic PXA. The multivariate analysis again showed that + 7, − 10q, and *TERT*p mutation were independent prognostic factors of OS (Table 3). One or more of these factors were detected in 22 of the 41 cases (54%). The median OS for these cases was 18.5 months, compared with 54.4 months for the cases without any of these factors ($P < 0.001$; Fig. 3d).

Discussion

Whereas 2016 revision of the WHO classification defines *IDH*-mutant grade II–III gliomas according to their molecular signature, it describes *IDH*-wt LGGs as an aggregate of several types of clinically and genetically distinct tumors without mutant *IDH*. However, several molecular features of *IDH*-wt LGGs have been proposed as predictors of prognosis [2, 3, 5–8, 29], but the candidate genetic alterations related to poor prognosis have differed between the reports: Wijnenga et al. and van den Bent et al. identified

Table 2 The results of the univariate analysis of OS according to clinical, histopathological and genetic factors in *IDH*-wild-type grade II–III gliomas

Factor	Group	n	Median OS (95% CI) (month)	*P* value
Age group (years)	< 50	9	32.8 (9.2–NA)	0.082
	≥ 50	26	24.6 (15.1–39.5)	
Sex	Female	15	24.6 (9.1–30.0)	0.105
	Male	20	39.5 (15.9–54.5)	
KPS	< 80	13	24.6 (5.9–NA)	0.134
	≥ 80	22	30 (15.9–54.5)	
Enhancement on MRI	Yes	25	20.9 (12.7–30)	0.087
	No	10	39.5 (18.5–NA)	
Extent of resection	GTR	14	20.9 (9.2–32.8)	0.425
	STR	8	35.7 (15.9–NA)	
	PR/biopsy	13	24.6 (5.9–NA)	
Adjuvant therapy	Chemotherapy	28	24.6 (15.9–32.8)	0.073
	None	7	NA (9.1–NA)	
Adjuvant therapy	Radiation therapy	26	29.7 (15.9–39.5)	0.825
	None	9	28.9 (3.6–NA)	
Histopathological diagnosis	DA	8	41.4 (18.5–NA)	0.031*
	AA	27	24.6 (12.7–30.0)	
Histopathological calcification	Yes	4	18.5 (16.6–NA)	0.742
	No	31	29.7 (15.1–39.5)	
MIB-1 index	High	17	29.7 (12.7–NA)	0.717
	Low	18	28.9 (15.1–41.4)	
Chromosome 7	+ 7	15	20.9 (9.1–32.8)	0.011*
	7 intact	20	41.4 (15.9–NA)	
Chromosome 10	− 10q	5	10.3 (5.9–NA)	0.01*
	10q intact	30	29.7 (16.6–41.4)	
*TERT*p	C228T or C250T	5	15.9 (3.6–NA)	0.034*
	wt	29	30.0 (18.5–41.4)	
ATRX	mut	6	24.6 (3.6–NA)	0.977
	wt	29	29.7 (15.1–41.4)	
BRAF	V600E	1	NA (NA)	0.862
	wt	34	28.9 (16.6–39.5)	
H3 F3A	K27M	1	28.9 (NA)	0.69
	wt	34	29.7 (15.9–39.5)	

AA anaplastic astrocytoma, *ATRX alpha-thalassemia/mental retardation syndrome, X-linked*, *BRAF B-rapidly accelerated fibrosarcoma*, *CI* confidence interval, *DA* diffuse astrocytoma, *GTR* gross total resection, *H3F3A histone 3 family 3A*, *IDH isocitrate dehydrogenase*, *KPS* Karnofsky Performance Scale score, *MRI* magnetic resonance imaging, *mut* mutation, *NA* not applicable, *PR* partial resection, *OS* overall survival, *STR* subtotal resection, *TERTp telomerase reverse transcriptase* promoter, *wt* wild-type, *+ 7* gain of chromosome 7, *− 10q* loss of chromosome 10q

*$P < 0.5$

Table 3 Summary of the multivariate analysis of OS for the 35 cases excluding those with pleomorphic xanthoastrocytoma and for all 41 cases

Factor	Group	OS ($n = 35$ cases)		OS ($n = 41$ cases)	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Chromosome 7	+ 7	5.00 (1.47–16.67)	0.01*	4.35 (1.35–14.29)	0.013*
Chromosome 10	− 10q	12.5 (2.38–50.00)	0.002*	14.29 (3.45–50.00)	< 0.001*
*TERT*p	C228T or C250T	7.69 (1.43–50.00)	0.018*	12.5 (2.17–100.00)	0.004*

CI confidence interval, *HR* hazard ratio, *OS* overall survival, *TERTp telomerase reverse transcriptase* promoter, *+ 7* gain of chromosome 7, *− 10q* loss of chromosome 10q

*$P < 0.05$

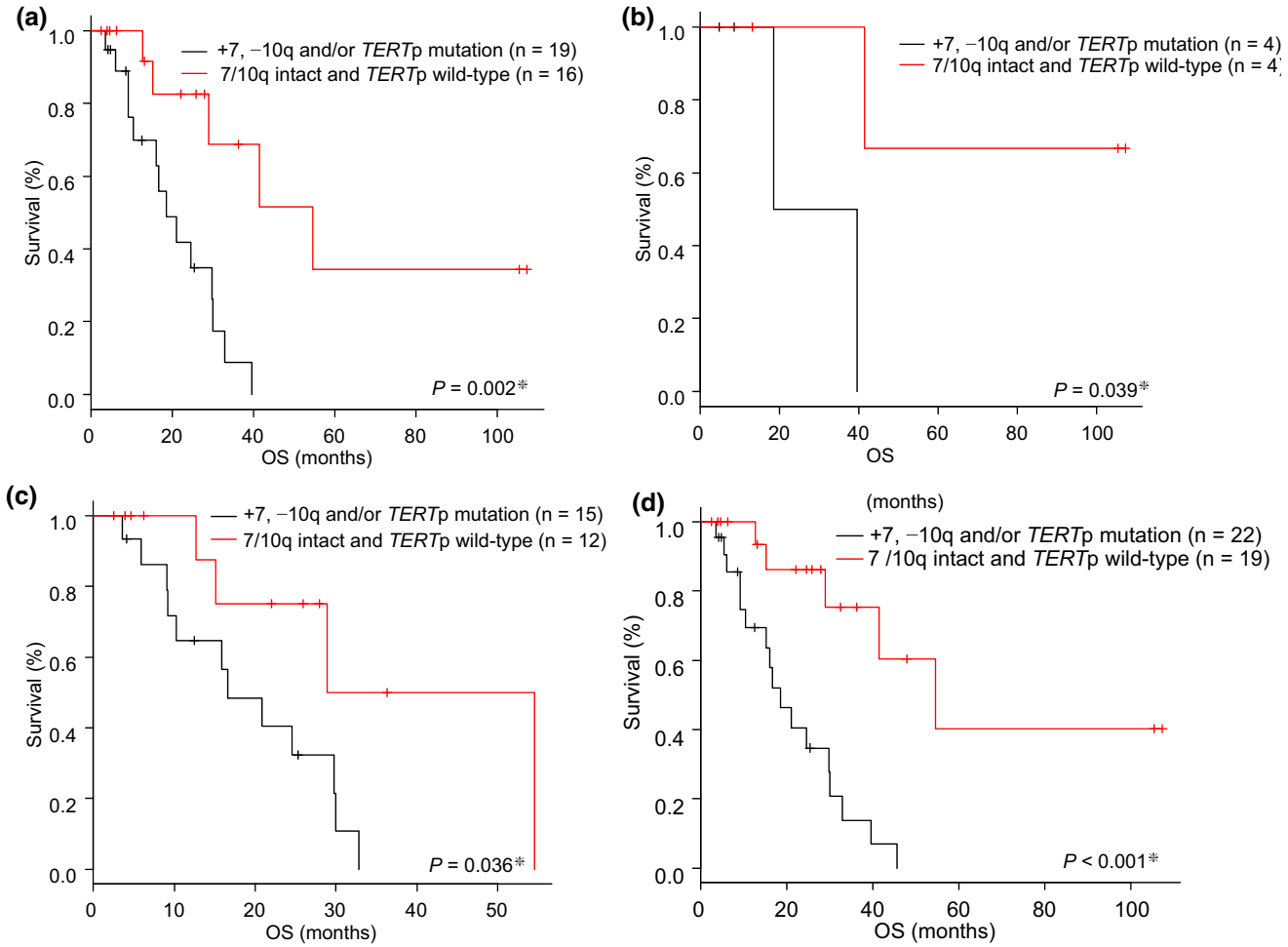

Fig. 3 Kaplan–Meier analysis of OS for *IDH*-wild-type lower-grade gliomas, excluding the pleomorphic xanthoastrocytoma (PXA) phenotype (**a**–**c**) and including the PXA phenotype (**d**), comparing the cases with tumors harboring tumor + 7, − 10q, and/or *TERT*p mutation with those that did not. **a** All 35 cases without PXA; **b** WHO grade II gliomas; **c** WHO grade III gliomas; **d** all 41 cases including PXA. *OS* overall survival, *TERTp telomerase reverse transcriptase* promoter, + 7 gain of chromosome 7, − *10q* loss of chromosome 10q. *$P < 0.05$

the prognostic value of + 7/− 10q and *TERT*p mutation [2, 8]; Aibaidula et al. proposed *EGFR* amplification, *TERT*p mutation, and *H3F3A* [3]; Aoki et al. proposed the gain of chromosome 7p, − 10q, and *TERT*p mutation [7]; and Stichel et al. and Brat et al. proposed *EGFR* amplification, + 7/− 10, and *TERT*p mutation [5, 6]. All of these reports identified *TERT*p mutation as a prognostic factor; however, there was inconsistency over the prognostic value of *EGFR* amplification and the combinations of total or partial gain of chromosome 7 and total or partial loss of chromosome 10.

Stichel et al. reported that *IDH*-wt LGGs with + 7/− 10, gain of chromosome 7q (+ 7q)/− 10, and + 7/− 10q were associated with poor OS and that the clinical course of *IDH*-wt LGGs with these copy number aberrations was similar to those of *IDH*-wt glioblastomas [5]. Their report defined the combinations of + 7/− 10, + 7q/− 10, and + 7/− 10q as the "7/10 signature." *EGFR* amplification was observed in 36% of *IDH*-wt glioblastomas, whereas *TERT*p mutation and the 7/10 signature were observed in 67% and 59% of *IDH*-wt glioblastomas, respectively [5]. *EGFR* amplification without *TERT*p mutation or the 7/10 signature was detected in only 15 of the 544 cases (2.8%) of *IDH*-wt glioblastomas [5]. Given this low frequency of *EGFR* amplification without *TERT*p mutation or the 7/10 signature and the complexity of assessing *EGFR* amplification, the evaluation of *EGFR* amplification was omitted from the present study.

When we divided our cases into two groups according to whether or not they showed at least one of the three significant prognostic factors (+ 7, − 10q, and/or *TERT*p mutation), the median OS of the groups was found to be significantly different, despite 35 cases, indicating that this subclassification was useful for the prediction of prognosis. In previous reports, the analyses of *IDH*-wt LGGs excluded the PXA phenotype [3, 4, 7, 9, 10]. In the main analysis of

the present study, we analyzed the 35 cases of *IDH*-wt DA and *IDH*-wt AA, excluding the cases of PXA or anaplastic PXA. However, the histopathological diagnosis of PXA can sometimes be confused with that of astrocytoma. We, therefore, reanalyzed the data, including the six cases with PXA or anaplastic PXA. This again showed the same three significant prognostic factors, demonstrating the usefulness of our subclassification regardless of whether cases of PXA and anaplastic PXA were included.

None of the cases of *IDH*-wt LGG in this study harbored + 7, − 10q, and *TERT*p mutation together; however, all three genetic alterations were reported in about 50% of glioblastoma cases [5]. This suggests that all *IDH*-wt LGGs with poor prognostic factors are not merely underdiagnosed *IDH*-wt glioblastomas. The combination of all three genetic alterations might tend to result in the microvascular proliferation and palisading necrosis characteristic of glioblastoma.

In summary, this study of cases of WHO grade II or grade III tumors showed that the patients with tumors harboring one or more of the genetic alterations + 7, − 10q, and/or *TERT*p mutation had shorter OS compared with the patients without these alterations. The molecular subclassification used in this study was more useful than WHO grade for predicting the prognosis of *IDH*-wt LGGs. This genetic subclassification could allow patients with poor prognostic factors to be identified, without delusion by histology, and given appropriate treatment. In our cases, the median OS for the cases of *IDH*-wt DA harboring + 7, − 10q, and/or *TERT*p mutation were extremely short compared with that for the other DA cases, as previously reported [29].

Our study suggested the potential subgroups within the group harboring + 7, − 10q, and/or *TERT*p mutation: a group with only + 7 and a group with − 10q and/or *TERT*p mutation. Although cIMPACT-NOW update 3 recommended the analysis of the combinations of chromosome 7 and 10, *EGFR* amplification and *TERT*p mutation, and divided *IDH*-wt LGGs into two groups, it might be better to differ tumors with only + 7 from other tumors with poor prognostic factors.

This was the first study to evaluate the usefulness of molecular information for the prediction of prognosis among Japanese patients with *IDH*-wt LGG at a single institution [2–5, 7, 30]. Although the number of cases was not large, the results may be informative. The findings suggested that *IDH*-wt LGGs could be divided into two groups with different clinical courses. However, it is unclear whether *IDH*-wt LGGs with poor prognostic factors are identical to *IDH*-wt glioblastomas; further research is needed to establish this.

In conclusion, this study showed that *IDH*-wt LGG could be subclassified into two groups in terms of prognosis according to the status of chromosome 7, the q arm of chromosome 10, and *TERT*p mutation. However, the wider application of genetic information requires the development of a method to check molecular information more easily, which could allow patients with a poor prognosis to be identified and treated appropriately.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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