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Genotyping low-grade gliomas among Hispanics

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Background. Low-grade gliomas (LGGs) are classified by the World Health Organization as astrocytoma (DA), oligodendroglioma (OD), and mixed oligoastrocytoma (OA). TP53 mutation and 1p19q codeletion are the most-commonly documented molecular abnormalities. Isocitrate dehydrogenase (IDH) 1/2 mutations are frequent in LGGs; however, IDH-negative gliomas can also occur. Recent research suggests that ATRX plays a significant role in gliomagenesis.

Methods. We investigated p53 and Olig2 protein expression, and *MGMT* promoter methylation, 1p19q codeletion, IDH, and ATRX status in 63 Colombian patients with LGG. The overall survival (OS) rate was estimated and compared according to genotype.

Results. The most common histology was DA, followed by OD and OA. *IDH1/2* mutations were found in 57.1% and MGMT+ (positive status of MGMT promoter methylation methyl-guanyl-methyl-transferase gene) in 65.1% of patients, while overexpression of p53 and Olig2 was present in 30.2% and 44.4%, respectively, and 1p19q codeletion in 34.9% of the patients. Overexpression of ATRX was analyzed in 25 patients, 16% tested positive and were also mutations in isocitrate dehydrogenase and negative 1p19q-codelition. The median follow-up was 15.8 months (95% CI, 7.6–42.0) and OS was 39.2 months (95% CI, 1.3–114). OS was positively and significantly affected by MGMT+, 1p19q codeletion, surgical intervention extent, and number of lobes involved. Multivariate analysis confirmed that MGMT methylation status and 1p19q codeletion affected OS.

Conclusions. This is the first study evaluating the molecular profile of Hispanic LGG patients. Findings confirmed the prognostic relevance of MGMT methylation and 1p19q codeletion, but do not support *IDH1/2* mutation as a relevant marker. The latter may be explained by sample size and selection bias. ATRX alterations were limited to patients with DA and were mutations in isocitrate dehydrogenase and negative 1p19q-codelition.

Keywords: biomarker, Hispanics, low-grade gliomas, molecular profile, prognosis.

The World Health Organization (WHO) classifies adult gliomas into 3 major groups according to the presumptive cell type of origin: astrocytoma (DA), oligodendroglioma (OD), and mixed oligoastrocytoma (OA). Specific signs of anaplasia (including mitosis, nuclear atypia, cell density, microvascular proliferation, and necrosis) further distinguish gliomas into grade II (low-grade glioma [LGG]), III (anaplastic), and IV (glioblastoma, GBM).[1] LGG categories include: subependymal giant cell astrocytoma, pilocytic astrocytoma, pilomyxoid astrocytoma, diffuse astrocytoma, pleomorphic xanthoastrocytoma, OD, OA, and some ependymomas.[2] These tumor subtypes account for approximately 10% to 20% of primary brain tumors and affect mainly young adults.[3] There are currently no epidemiological data for these tumors in Colombia.

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Histological characteristics (eg, necrosis, mitotic activity, nuclear atypia, and proliferative index) are the main clues for the diagnosis, prognosis, and management of these tumors. However, since the late 1990s different molecular abnormalities have been identified as supportive markers for diagnostic, prognostic, and treatment purposes. These molecular surrogates include isocitrate dehydrogenase (IDH) mutations, O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation status, deletions involving chromosomes 1p and 19q, TP53 mutations, and v-raf murine sarcoma viral oncogene homolog B1mutations (BRAF).[3]

Mutations in the isocitrate dehydrogenase 1 and 2 (*IDH1/2*) genes distinguish grade II, III, and secondary GBM from primary GBM.[4–6] *IDH1/2* mutations have been recorded in up to 85% of LGGs.[7] The absence of IDH mutation (wild type) identifies a novel radiological and molecular subtype of LGG with dismal prognosis.[8] Moreover, two additional genetic alterations have been described in grade II and III gliomas: TP53 mutations, which characterize astrocytomas, and the 1p19q codeletion (the result of a t(1;19)(q10;p10) translocation), documented in OD.[9] It is recognized that there is a significant association between 1p19q codeletion and better prognosis, while the opposite is true for TP53 mutations.[10–12] Similarly, Kim et al, suggested that 1p19q-codeleted (1p19q+) tumors conferred a better prognosis than TP53-mutated tumors in a large group of LGGs, regardless of IDH status. Furthermore, *MGMT* promoter methylation was more prevalent in LGGs and was linked with a better prognosis and response to the alkylating agent temozolomide.[13–15]

Even though mixed gliomas are known to have variable outcomes, they share common genetic alterations with both OD and OA; for example OA also carry either a TP53 mutation (~40%) or a 1p19q codeletion (~45%)[11] It is highly likely that TP53 mutation (p53+) and 1p19q codeletion are mutually exclusive and involve IDH-mutated (IDH+) glial precursor cells; however, we are still waiting for further experimental evidence.[11,12]

According to the IDH, TP53, and 1p19q status, four major subtypes of LGGs have been characterized: IDH+/p53-/1p19q-, IDH+/p53+/1p19q-, IDH+/p53-/1p19q+, and triple negative, with triple negative having the worst prognosis.[9] Mutation of the α-thalassemia/mental retardation syndrome X-linked (*ATRX*) gene and loss of ATRX protein expression, detected by immunohistochemistry, have been described in gliomas of various subtypes and grades.[16–20] ATRX enhances histone 3.3 variant incorporation into heterochromatin, giving rise to telomere-length changes and genomic instability.[21–23] A significant correlation was also identified with alternative lengthening of telomeres.[24,25] In adult gliomas, this alteration was more prevalent in astrocytic tumors than mixed glial tumors, while it was rare in pure ODs. Additionally, mutations in the *ATRX* gene were strongly associated with IDH and TP53 mutations.[18–21]

In the present study, we clinically, pathologically, and molecularly characterize a group of Hispanic patients with LGG who were treated in a single institution in Colombia. We further discuss the association between the mentioned molecular features with prognosis and overall survival.

Materials and Methods

This study was designed to assess p53 and Olig2 protein expression, MGMT methylation status, 1p19q codeletion, and IDH/ATRX status in 63 Colombian LGG patients. The purpose was to correlate these results with multiple outcomes, including progression-free survival (PFS) and overall survival.

Patient Characteristics and Tissue Samples

Adult (>18 years old) patients were recruited prospectively from a single institution in Bogotá, Colombia. Demographic, clinical (ie, symptoms and signs), radiological characteristics, and survival data (ie, overall survival and PFS) were collected. Samples of LGG specimens, obtained via surgical biopsy or resection, were retrieved from the archives at the Department of Pathology of *Fundación Santa Fé de Bogotá and Foundation for Clinical and Applied Cancer Research*. Archived tissue specimens were selected by two independent pathologists according to histological WHO classification of gliomas (diffuse astrocytoma, OD, or OA),[1] and their agreement was 94%. Written informed consent was obtained from all participants. A local ethics committee approved the use of brain tumor tissue and clinical data for research purposes.

Direct DNA Sequencing of IDH1/2 Mutations

Genomic DNA was isolated from the surgical specimens using a Qiagen kit (Qiagen, Valencia, CA, USA). PCR primers were designed for the genomic region corresponding to the portion of *IDH1* exon 4 that encodes codon R132, as follows: *IDH1* sense (5′-AAACAAA TGTGGAAATCACC-3′) and *IDH1* antisense (5′-TGCCAACATGACTTAC TTGA-3′). The PCR conditions were 94°C for 5 minutes; 36 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute; and extension at 72°C for 5 minutes. PCR was performed using Ex-Taq HS DNA Polymerase (Takara Bio, Shiga, Japan). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen), according to the manufacturer's instructions. Sequencing reactions were performed using previously described primers, a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA), and an ABI377 automated sequencer (Applied Biosystems). 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.

Methylation-specific PCR for MGMT Promoter

MGMT methylation was detected using methylation-specific PCR (MSP). Genomic DNA from each sample (2 μg) was treated with sodium bisulfite using the Epitect Bisulfite Kit (Qiagen Valencia, CA). The primer sequences for the unmethylated reactions were 5′-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3′ (forward) and 5′-AACTCC ACACTCTTCCAAAAACAAAACA-3′ (reverse), and those for the methylated reaction were 5′-TTTCGACGTTCGTAGGTTTTCGC-3′ (forward) and 5′-GCACTCTTCCGAAAACGAAACG-3′ (reverse). The PCR conditions were as follows: 95° for 5 minutes; 34 cycles of 95° for 30 second, 61° for 30 second, 72° for 30 second; and extension at 72° for 4 minutes. Amplified products were separated on 3% agarose gels, stained with ethidium bromide, and visualized under UV illumination.

1p/19q Co-deletion Analysis by Fluorescence in Situ Hybridization

Fluorescence in situ hybridization (FISH) was performed according to standard methods. Control and detecting probes were developed from the plasmids D1Z1 (1q12) and D1Z2 (1p36.3),

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respectively, for the chromosome 1 study and from bacterial artificial chromosomes (BACs) RP11-413 M18 (19q13) and CTZ-2571 L23 (19q13.3), respectively, for the chromosome 19 study. Different colored probes were used to detect chromosomal loss at chromosomes 1p and 19q: a single fluorescent signal in the nucleus was interpreted as chromosomal-arm loss if two signals were detected for the control probe.

ATRX

Immunohistochemistry for ATRX was performed using a polyclonal rabbit antibody (dilution 1:400, product code HPA001906, Sigma Aldrich, St. Louis, MO, USA), an automated immunostainer (Benchmark Ultra, Ventana, Tucson, AZ, USA) and standard protocols, including pretreatment with Cell Conditioning 1 buffer (Ventana) for 52 minutes and standard Ventana signal amplification methods. DNA was isolated, and the entire coding sequence of ATRX was analyzed by Sanger sequencing.

TP53 Immunostaining

Immunostaining for TP53 was performed using the monoclonal antibody DO-7 (Dako IR616) on a BenchMark XT (Ventana Medical Systems, Tucson, AZ) automatic staining system, according to the manufacturer's protocol. Tissue samples were counterstained with hematoxylin. TP53 nuclear positivity was defined by the number of cells with positive immunohistochemistry in vital tumor areas, excluding perinecrotic areas, which often show some degree of (hypoxia-associated) TP53 immunoreactivity. The cutoff for TP53 immunopositivity was defined as $\geq$20% of positive cells, whereas tumors with $\leq$19% positive cells were considered negative. Magnification was 100×. Two board-certified neuropathologists performed these quantitative assessments.

Olig2 Immunostaining

Immunostaining for Olig2 was performed using a polyclonal Olig2 antibody (dilution 1:300; Chemicon-Millipore Corp.) on a BenchMark XT (Ventana Medical Systems, Tucson, AZ) automatic staining system, according to the manufacturer's protocol. Olig2 is a nuclear protein. Nuclear staining was therefore considered positive. Sections with known positivity from an OD grade II tumor case were added to each batch of slides as a positive control. The absence of a primary antibody was used as a negative control. Counting was carried out in 10 areas in each case (original magnification ×400). Positive cells were scored semiquantitatively as follows: Score 0 (no expression) = 0% to 4% of cells were positive; Score 1 (weak expression) = 5% to 24% of cells were positive; Score 2 (moderate expression) = 25% to 50% of cells were positive; Score 3 (strong expression) = >50% of cells were positive. For statistical purposes, tissue samples with a score of 2 or 3 were considered to be positive.

Statistical Analysis

For descriptive purposes, continuous variables were summarized as arithmetic means, medians, and standard deviations. Categorical variables were reported as proportions with 95% confidence intervals (95% CI). Inferential comparisons were performed using Student's *t*-test, chi-square and Fisher's exact tests were used to assess significance between categorical variables. The time-to-event variables obtained from the Kaplan-Meier method were determined by log-rank tests. Statistical significance was determined as $P \leq .05$ with a two-sided test. The agreement between pathologists was estimated using kappa. All statistical analysis was performed with SPSS version 19.0 (SPSS, Inc., Chicago, IL, US).

Results

Sixty-three adult patients with LGG treated at an academic institution from Colombia were selected. Table 1 describes the demographic and clinical characteristics of the study population. The median age was 40 years and patients were evenly distributed by gender. The most common histology was DA, observed in

Table 1. Baseline demographic, clinical, and histological characteristics of the study population

Variable	$N = 63$ (%)
Sex	
Female	32 (50.8)
Male	31 (49.2)
Age, mean	40.1 (±12.3)
<40 years	35 (55.6)
>40 years	28 (44.4)
Histology	
Astrocytoma	39 (61.9)
Oligodendroglyoma	16 (25.4)
Mixed oligoastrocytoma	8 (12.7)
Type of surgery	
Total	30 (47.6)
Subtotal	20 (31.7)
Biopsy	13 (20.6)
Neurological symptoms	
Minor symptoms	54 (85.7)
Greater neurological deficit	9 (14.3)
Seizures	
Yes	9 (14.3)
No	54 (85.7)
Number of lobes involved	
One	45 (71.4)
Two or more	18 (28.6)
Cross the midline	
Yes	6 (9.5)
No	57 (90.5)
Tumor diameter	
Mean (mm)	41.7 (SD±17.2)
<5 cm	38 (60.3)
>5 cm	16 (25.4)
Treatment after surgery	
Radiotherapy	20 (31.7)
Chemotherapy (temozolomide)	12 (19.0)
Watch and wait	31 (49.2)
Grade change during follow-up (by images or biopsy)	
Yes	24 (38.1)
No	39 (61.9)
Vital status at last control	
Alive	54 (85.7)
Dead	9 (14.3)

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61.9% of the study population, which had a median lesion size of 50 mm. The median follow-up was 15.8 months (95% CI, 7.6–42.0). Characteristics associated with worse prognosis were common in the cohort: 44% of LGG patients were over 40 years old, 14% had significant neurological symptoms, 9.5% of LGG lesions crossed the midline, and 28% of LGG lesions involved two or more lobes (Table 1).

Most patients ($n = 30$, 47.6%) underwent total resection, while 20 (31.7%) had subtotal resection. Surgical biopsy was performed in 13 patients (20.6%) (Supplementary material, Table S1). Patients who underwent total resection were observed closely and received radiation therapy at first recurrence, while patients who underwent subtotal resection had adjuvant radiation. TMZ was administered to 12 patients who received in average 12 cycles (SD = 1.28), while TMZ and radiation therapy was given to 8 patients (12%) (Supplementary material, Tables S2 and S3). In Figure 3 we illustrate overall survival by resection extent; patients who underwent subtotal resection had the highest overall survival, followed by total resection and biopsy ($P = .012$). Supplementary material, Table S4 discriminate genomic alterations among included patients.

IDH1/2 Mutations

Thirty-six (57.1%) patients in the study population had *IDH1/2* mutations (Table 2). The presence of *IDH1/2* mutations (IDH+) was not related to clinical characteristics such as age, gender, or lesion size. As expected, IDH+ was most frequently observed in patients with ODs (16 IDH mutations in 16 oligodendrogliomas = 100%); $P = .001$). IDH+ was significantly more frequent in patients with DA ($n = 13$, 36.1%) than in patients with OA ($n = 7$, 19.4%) (Table 3).

We found a positive relationship between *IDH1/2* mutations and other biological characteristics, including 1p19q codeletion ($P = .001$), Olig2, and p53 overexpression. However ATRX expression was not related to the presence of *IDH1/2* mutations (Table 3).

MGMT Methylation

MGMT methylation was observed in 41 patients (65.1%) (Table 2). MGMT+ was more common in patients with DA and OA histology (43.9% and 39.9%, respectively), while it was only present in 17.1% of patients with OD ($P = .001$). Neither gender nor age influenced MGMT methylation.

We observed that 1p19q codeletion and p53 overexpression were associated with MGMT methylation; 76% of patients who were MGMT+ also had 1p19q codeletion. MGMT methylation could possibly be explained as a result of G-CIMP by IDH1 mutation. We also documented that patients with p53 overexpression consistently had MGMT methylation ($P = .001$). Conversely, Olig2-positive patients frequently lacked MGMT methylation ($P = .002$) (Table 3).

1p19q Codeletion

1p19q codeletion was analyzed in 56 patients; 22 patients (34.9%) tested positive (1p19q+) (Table 2). 1p19q+ was more frequent in OD (63% of 1p19q+) than in other tumor histologies. After analyzing the relationship between 1p19q+ and other biological characteristics, we found that 1p19q+ was associated with *IDH1/2* mutations ($P = .001$) and p53 overexpression ($P = .003$). On the other hand, 1p19q+ was not related to ATRX expression.

Table 2. Tumor molecular profiling

Variable	$N = 63$
ATRX expression	($N = 25$)
Positive	4 (16.0)
Negative	21 (84)
***IDH mutation* status**	
Positive	36 (57.1)
Negative	26 (41.3)
Not determined	1 (1.6)
***MGMT methylation* status**	
Positive	41 (65.1)
Negative	21 (33.3)
Not determined	1 (1.6)
P53 protein expression	
Positive	19 (30.2)
Negative	42 (66.7)
Not determined	2 (3.2)
1p19q codeletion	
Positive	22 (34.9)
Negative	34 (54.0)
Not determined	7 (11.1)
Olig2 expression	
Positive	28 (44.4)
Negative	35 (55.6)
Ki67	
0%–10%	29 (46.0)
11%–20%	13 (20.6)
>20%	13 (20.6)
Not determined	8 (12.7)

ATRX Expression

Due to economic constraints and monoclonal import processes, ATRX expression was only analyzed in 25 patients. Twenty-one patients (84%) were negative for ATRX expression (Table 2). Our sample size limited the analysis of the relationship between positive ATRX expression and clinical characteristics. However, the four ATRX+ patients had DA ($P = .125$) and closely overlapped with *IDH1/2* mutations ($P = .125$).

Distribution According to Molecular Subgroups

Following the classification suggested by Figarella-Bragner et al,[9] which is based on IDH, TP53, and 1p19q status, the most common subgroup encountered was triple negative (IDH-/TP53-/1p19q-; 33%), followed by triple positive and IDH+/p53-/1p19q+ (19% and 15.9%, respectively). Table 4 describes the distribution of patients according to molecular subgroups.

Overall Survival

Overall survival was 39.2 months (95%CI 1.3–114) (Fig. 1). Patients with OD or OA had better survival times compared with patients with DA histology ($P = .01$) (Fig. 2A). No additional clinical features affected overall survival (Fig. 2B and C). 1p19q codeletion

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Table 3. Characteristics of patients according to *IDH/MGMT* methylation status

Variable	IDH Status		*P*	MGMT Status		*P*
	IDH+	IDH−		Positive	Negative	
Age						
<40 years	23 (67.6)	11 (32.4)	.07	25 (73.5)	9 (26.5)	.13
>40 years	13 (46.4)	15 (53.6)		16 (57.1)	12 (42.9)	
Sex			.62			.5
Male	18 (58.1)	13 (41.9)		21 (67.7)	10 (32.3)	
Female	18 (58.1)	13 (41.9)		20 (65.5)	11 (35.5)	
Diameter						
<5 cm	24 (51,0)	13 (49,0)	.13	27 (72.9)	10 (26.1)	.04
>5 cm	7 (43.8)	9 (56.2)		7 (43.8)	9 (56.2)	
Not determined	–	4 (100)		7 (100)	–	
Histology						
Astrocytoma	13 (34.2)	25 (65.8)	.001	18 (47.4)	20 (52.6)	.0001
Oligodendroglioma	16 (100)	–		7 (87.5)	1 (12.5)	
Oligoastrocytoma	7 (87.5)	1(12.5)		16 (100)	–	
1p19q status						
Positive	22 (100)	–	.001	22 (57.9)	16 (42.1)	.001
Negative	12 (64.7)	22 (35.3)		18 (100)	–	
Not determined	–	–		1 (16.6)	5 (83.4)	
ATRX status						
Positive	4 (100)	0	.1	3 (60.0)	1 (40.0)	.23
Negative	11 (52.4)	10 (47.4)		15 (71.4)	6 (28.6)	
Not determined	–	–		23 (62.2)	14 (37.8)	
Olig2						
Positive	21 (75.0)	7 (25.0)	.01	25 (89.3)	3 (10.7)	.002
Negative	15 (44.1)	19 (55.9)		16 (66.7)	18 (33.3)	
P53						
Positive	18 (94.7)	1 (5.3)	.001	18 (48.6)	19 (51.4)	.001
Negative	17 (40.5)	25 (59.5)		23 (100)	–	

Table 4. Molecular subgroups

Molecular subgroup	Frequency	%
IDH+/p53-/1p19q-	7	11.1
IDH+/p53-/1p19+	10	15.9
IDH+/p53+/1p19q-	5	7.9
IDH+/p53+/1p19q+	12	19
Triple negative	21	33.3
Not determined	8	12.7
Total	63	100

status modified overall survival, given that 1p19q+ patients had a better prognosis than 1p19q- patients (median not reached vs 44.2 months; $P = .01$) (Fig. 3A). MGMT methylation status also influenced prognosis, since MGMT+ patients had a better prognosis than MGMT-negative patients (median 110.8 vs 52.3 months; $P = .01$) (Fig. 3B). In multivariate analysis, 1p19q and MGMT methylation also altered overall survival ($P = .047$ and $P = .039$, respectively).

None of the remaining molecular characteristics, including *IDH1/2* mutations, modified overall survival. However, when we combined *IDH1/2* mutation status with *MGMT* promoter methylation status, we found that double-positive (IDH1/ 2+, MGMT+) patients had better survival rates (median not reached) than double-negative (IDH1/2-, MGMT-) patients (22.4 months, $P = .0001$) (Fig. 4).

Discussion

To our knowledge this study is the first attempt to establish the molecular profile and prognosis in a cohort Hispanic patients with LGG. We found that MGMT methylation status and 1p19q codeletion modified overall survival. Specifically, patients with methylation of the *MGMT* gene promoter had better overall survival rates compared with patients without methylation, moreover patients with 1p19q codeletion had a better prognosis. This suggests that these molecular features are independent prognostic factors.

The prognostic impact of 1p19q codeletion in LGG is not entirely clear because inclusion criteria vary across studies that follow different LGGs as well as anaplastic gliomas.[26–33] So far, the 1p19q codeletion has not been related to prognosis in patients who are not candidates for adjuvant treatment; however, given the association between 1p19q codeletion and responsiveness

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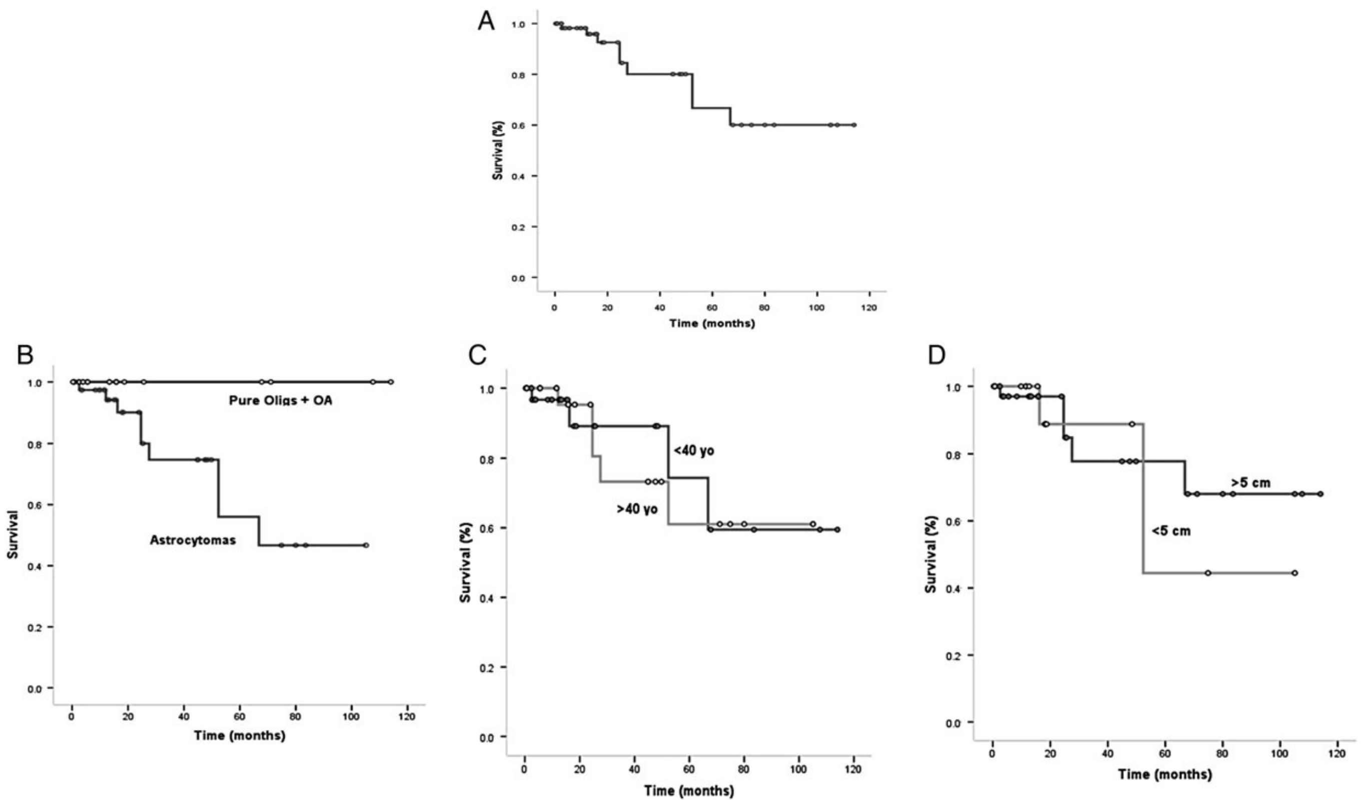

Fig. 1. Overall survival (A) and survival curves based on histology (B), age (C), and tumor diameter (D). Abbreviations: Oligs, Oligodendroglioma; OA, Oligoastrocytomas.

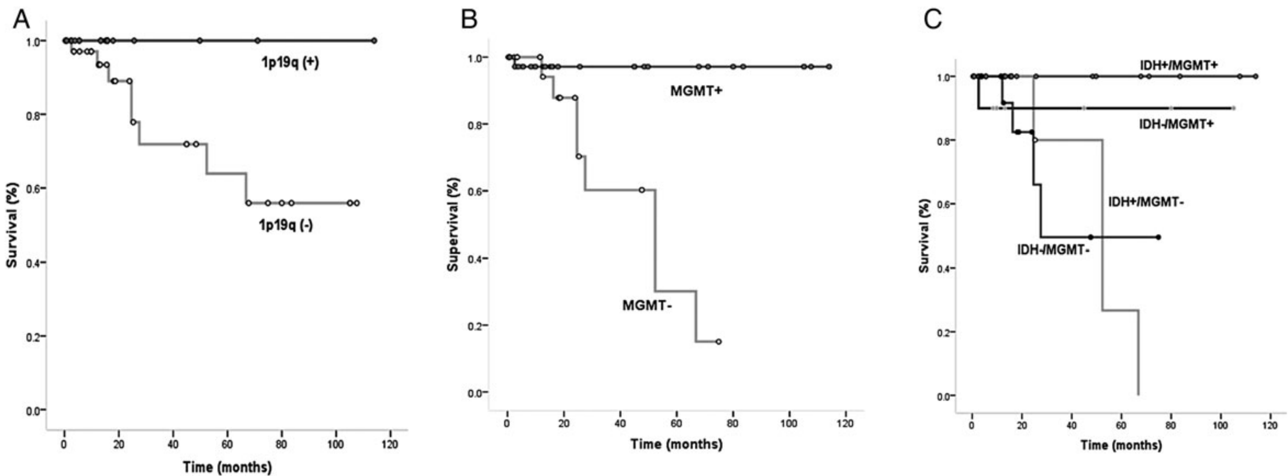

Fig. 2. Overall survival based on molecular profile: 1p19q status (A), MGMT methylation status (B), *IDH1/MGMT* methylation status (C).

to DNA damage treatments, some authors have suggested that 1p19q is a predictive rather than a prognostic factor for tumor responsiveness to radiotherapy and chemotherapy.[34–36] In our study, 1p19q codeletion was related to better overall survival independent of other clinical factors, including treatment. Similarly the Cancer Genome Atlas Research Network has recently published a comprehensive analysis of molecular signatures of LGG. The authors found that LGGs with an *IDH mutation* and 1p/19q codeletion were significantly associated with favorable outcomes.[37] In contrast, other recent studies with similar populations did not find an association between prognosis and 1p19q codeletion in adults with LGG.[9,38] Research with improved study design is warranted in order to clarify the predictive and prognostic implications of 1p19q codeletion.

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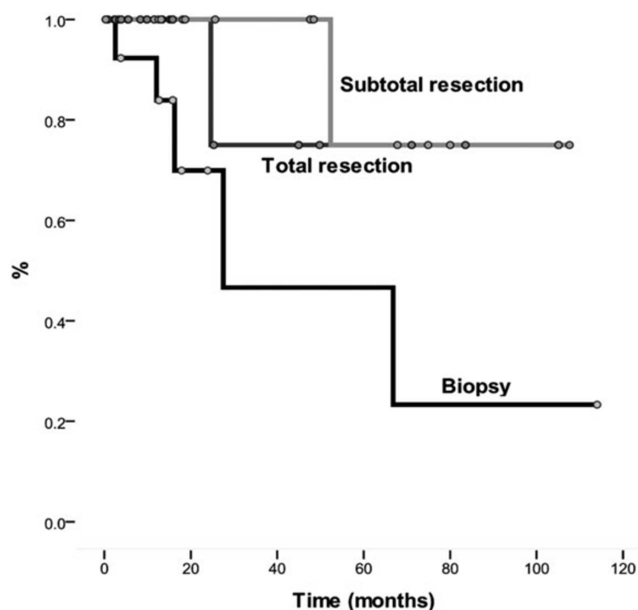

Fig. 3. Overall survival by resection extent.

Other than being associated with better overall survival, our results suggest that methylation of the *MGMT* gene was also related to negative 1p19q codeletion. This is consistent with similar results reported by Kesari et al[39] and Komine et al[40]; Kesari et al documented a very strong correlation between MGMT status and 1p19q codeletion, which is recognized as a probable prognostic factor for survival.[38,39] On the other hand, studies with multivariate analyses did not report an association between MGMT status and overall survival in patients with LGG.[40–44] The failure to demonstrate this association in some studies could possibly be avoided by implementing homogenous ultra sensitive techniques.

Although the prognostic value of *IDH1/2* mutations is widely recognized for malignant gliomas, the exact significance remains ambiguous. The German Glioma Network showed that *IDH1/2* mutational status was a strong prognostic marker for overall survival in a cohort of 139 patients with LGG, regardless of the histological type of the tumor. However, the relationship between *IDH1/2* mutational status and PFS was only reported in patients who received radiotherapy or chemotherapy at the time of diagnosis.[39] Other studies have supported the importance of IDH mutations by identifying a CpG island methylator phenotype in a subset of gliomas (G-CIMP), which is characterized by hypermethylation at different loci, including the *MGMT* promoter-associated CpG island.[40] Our study is consistent with previous research that documented that patients with *IDH1/2* wild-type LGG experience inferior outcomes.[8,45] Similarly, Noushmehr et al[41] reported that patients with G-CIMP were younger at the time of diagnosis and had significantly longer survival time.

In contrast, a large retrospective study of 360 LGG patients, established that *IDH1/2* mutations did not have prognostic significance.[13] Similarly, our study showed that isolated *IDH1/2* mutations did not affect overall survival. Sample size and selection bias may provide an explanation for our results. However, other authors have claimed that such contradictory results observed across studies of large groups of LGG patients is the pooling of different diffusely infiltrating LGG entities. This could possibly lead to an underestimation bias given the strong association between *IDH1/2* mutations and the favorable prognosis of 1p/19q codeletion in patients with WHO grade II ODs.

The Figarella-Bragner et al[9] retrospective study remarkably showed that the molecular subgroups of LGGs were independent prognostic factor in both univariate and multivariate analyses. In their study, the authors reported the following molecular signature distribution: 12.6% (10/79 patients) in group 1 (IDH+/p53-/1p19q-); 30.4% (24/79 patients) in group 2 (IDH+/p53-/1p19q+); 48.1% (38/79 patients) in group 3 (IDH+/p53+/1p19q-); and 8.9% (7/79 patients) in group 4 (triple negative). Additionally the authors reported that triple-negative patients had the worst survival [median 3.9 years (95% CI, 2.1–3.8)], whereas group 2 patients had the best survival [median 9.3 years (95% CI, 8.1–10.4)]. Interestingly, the incidence of triple-negative LGGs in our cohort was considerably higher. Possible hypotheses include exposure to environmental factors such as electromagnetic fields in Colombia from unregulated transmission mast sources or the recently described harmful association between triple negative tumors and microRNA dysregulation.[46]

Although prior studies have demonstrated that ATRX plays an important role in gliomagenesis through chromatin remodeling regulation and *IDH* mutations, the frequency of ATRX expression is still controversial. In agreement with Kannan et al, we determined that ATRX expression was positive in 16% (4/25) of patients and was exclusively present in patients with DA. Additionally, after analyzing ATRX expression with other molecular characteristics, ATRX expression was limited to IDH1/IDH2+ patients; this is also consistent with other studies that suggest that *ATRX-IDH1/2* phenotype is important in the early development and progression of astrocytic tumors.[37] We also acknowledged that ATRX expression was limited to 1p19q- patients. We did not find statically significant associations between ATRX+ status and clinical or molecular features. Moreover, there was no evidence of a relationship between ATRX expression and TP53 mutations, as opposed to the study of Jiao Y et al, which found that ATRX expression was related to the presence of TP53 mutations.[21] However, our results could be influenced by sample size bias. Only 25 patients were analyzed for ATRX expression and only 4 of those patients were positive.

As outlined in the Haarlem Consensus,[47] the upcoming WHO Classification will officially introduce molecular diagnosis as a part of the mandatory criteria. Even though this will change routine diagnostic practice in neuro-oncology, it is likely that the guideline's implementation in developing countries will be slow, due to several reasons. For example, in developing countries, formal neuropathology training, oncology education during medical school, and dissemination of updated information on brain tumors are all limited. However, in our region, the Latin American Neuro-Oncology Network - RedLANO holds an annual meeting on neuro-oncology, as an effort to create consensual management among health care providers (www.redlano.org). Another challenge that we continually face is the marginal investment in cancer research in Latin American countries.[48]

Our results suggest a remarkable similarity not previously described between the molecular profile of Hispanic patients with LGG and other populations. Additionally, we carried out a thorough molecular evaluation with treatment uniformity and long-term follow-up. However, our study has certain limitations.

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One such was that we did not correlate overall survival with molecular subgroups. Also, due to budget restrictions and test validation, we were not able to determine ATRX molecular expression in the entire study population. Further research will allow us to determine the expression of ATRX in low-grade astrocytoma.

Conclusions

Characterizing the molecular landscape of Hispanics is essential to understand the etiology and prognostic markers that can optimize targeted clinical management of LGGs. Our results confirmed the prognostic relevance of MGMT methylation status and 1p19q codeletion, but do not support a positive relationship with *IDH1/2* mutations. These findings may be due to sample size and selection bias. We also documented that the combination of MGMT methylation status and *IDH1/2* mutations influenced the prognosis of LGG in the study population.

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Disclaimer

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Conflict of interest statement. The authors declare no conflict of interest.

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