Preoperative Determination of Isocitrate Dehydrogenase Mutation in Gliomas Using Spectral Editing MRS: A Prospective Study

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Background: The edited magnetic resonance spectroscopy (MRS) technique has not yet been formally evaluated for the in vivo detection of 2-hydroxyglutarate (2-HG) in patients with gliomas of various grades.
Purpose: To evaluate the diagnostic accuracy of edited MRS in the preoperative identification of the isocitrate dehydrogenase (IDH) mutation status in patients with gliomas.
Study Type: Prospective.
Population: Fifty-eight subjects (31 glioblastomas, 27 grade II and III gliomas).
Field Strength/Sequence: Mescher–Garwood (MEGA)-PRESS and routine clinical brain tumor MR sequences were used at 3T.
Assessment: Data were analyzed using an advanced method for accurate, robust, and efficient spectral fitting (AMARES) from jMRUI software. The amplitudes of the 2-HG, N-acetyl-aspartate (NAA), choline (Cho), and creatine/phosphocreatine (Cr) resonances were calculated with their associated Cramer–Rao lower bound (CRLB). The IDH1 R132H mutation status was assessed by immunohistochemistry for all patients. Patients with grades II and III gliomas with negative immunohistochemistry underwent DNA sequencing to further interrogate IDH mutation status.
Statistical Test: The differences in 2-HG amplitudes, 2-HG/NAA, 2-HG/Cho, and 2-HG/Cr between IDH-mutant and IDH-wildtype gliomas were assessed using Mann–Whitney *U*-tests. Receiver operating characteristic curve analysis was performed to evaluate the diagnostic accuracy of each parameter.
Results: The 2-HG amplitudes, 2-HG/NAA, and 2-HG/Cho were higher for IDH-mutant gliomas than IDH-wildtype gliomas ($P < 0.007$). Using a CRLB threshold <30%, a 2-HG cutoff greater than 0 had a sensitivity of 80% (95% confidence interval [CI]: 52–96%) and a specificity of 81% (95% CI: 54–96%) in identifying IDH-mutant gliomas. In the subset of patients with grades II and III gliomas, the sensitivity was 80% (95% CI: 52–96%) and specificity was 100% (95% CI: 40–100%). Among 2-HG ratios, the highest AUC for the identification of IDH mutant status was achieved using the 2-HG/NAA (AUC = 0.8, 95% CI 0.67–.89).
Data Conclusion: Preoperative edited MRS appears to be able to help identify IDH-mutant gliomas with high specificity.
Level of Evidence: 1
Technical Efficacy Stage: 2

J. MAGN. RESON. IMAGING 2020.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/jmri.27366

Received Jun 20, 2020, Accepted for publication Aug 27, 2020.

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Contract grant sponsor: RSNA Research & Education Foundation: Research Seed Grant; Joan Sealy Trust: Internal Grant.

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THE MOLECULAR BIOLOGY of glioblastomas has recently revealed four subtypes, each with unique prognostic and biological properties.[1–3] Mutated isocitrate dehydrogenase (IDH) is a feature of the proneural subtype of glioblastoma and is associated with a favorable prognosis.[4,5] Lower-grade gliomas including oligodendrogliomas, diffuse infiltrating astrocytomas, and anaplastic astrocytomas also enrich for IDH mutations and belong to the proneural molecular subtype.[1,3] Mutations in the IDH1 or IDH2 genes result in the production of the onco-metabolite, 2-hydroxyglutarate (2-HG) from α-ketoglutarate.[2,6]

Preoperative knowledge of an IDH mutation might influence surgical decision-making. Beiko et al observed that surgical resection of nonenhancing disease prolonged overall survival in patients with malignant astrocytomas who have an IDH1 mutation and not in those without the mutation.[7] Magnetic resonance imaging (MRI) has been used preoperatively to predict the IDH status. On conventional MRI, the presence of the T_2/FLAIR (fluid-attenuated inversion recovery) mismatch sign has been reported to have high specificity but low sensitivity in the prediction of IDH mutation in low-grade gliomas.[8–10] Different MR spectroscopy (MRS) methods for detection of 2-HG have been evaluated in clinical practice.[11–15] Short-echo MRS can result in a high false-positive rate due to the spectral overlap of 2-HG with neighboring resonances.[11,12] Compared to short-echo MRS, long-echo MRS produces a narrower 2-HG signal at 2.25 ppm, which leads to improved differentiation between 2-HG and adjacent metabolites.[12,13] Edited MRS allows detection of the 2-HG peak at 4.02 ppm, as overlapping resonances from adjacent metabolites are removed from the difference spectrum.[14,15] There have been only a few studies using edited MRS to detect 2-HG in human subjects with glioma.[14,15] Thus, the purpose of this study was to evaluate the diagnostic accuracy of edited MRS in predicting the IDH mutation status in a consecutive series of patients who presented with a new glioma.

Materials and Methods

Study Population

This study was approved by the local Ethics Board (REB#20140754-01H). Written informed consent was obtained from each patient enrolled in this study. Patients with a newly suspected diagnosis of glioma between October 2015 and April 2019 at our hospital were enrolled. Inclusion criteria were: adult patient (≥18 years of age) with first lifetime presentation of suspected glioma on initial scans. Exclusion criteria were: non-surgical patients, prior temozolomide or brain radiation therapy, nonglioma tumors, patients with recurrent tumors, and inability to provide written informed consent. The age, sex, type of surgery, and time between MR exam and surgery were recorded for each patient.

MR Acquisition

All patients underwent a clinical examination with a 3T MR scanner (Trio, Siemens, Erlangen, Germany; VB17A) equipped with a 32-channel receive-only head coil. All studies were supervised by a neuroradiologist or an MR physicist. Coronal FLAIR (repetition time [TR] = 9710 msec, echo time [TE] = 90 msec, inversion time [TI] = 2580 msec), axial T_2–weighted (TR = 6700 msec, TE = 97 msec), axial T_1-weighted (TR = 280 msec, TE = 2.5 msec), and axial FLAIR (TR =9710 msec, TE = 93 msec, TI = 2580 msec) images were obtained for localization of the tumor before gadolinium administration. Spectroscopy was obtained before contrast administration.

The spectral editing sequence was based on the method published by Choi et al[11] using the Mescher–Garwood (MEGA)-PRESS editing technique.[16,17] Automatic voxel selective gradient shimming implemented in the Siemens spectroscopy package was performed before acquisition. If the linewidth (measured as full-width, half-maximum [FWHM]) of the unsuppressed water from the voxel exceeded 18 Hz, additional manual shimming by the MR physicist or an automatic shimming technique (fast automatic shim technique using echo-planar signal trains utilizing mapping along projections [FAST(EST)MAP]) was applied to improve the shim to a water linewidth <18 Hz.[18] FAST(EST)MAP shimming became available only later in the study and it was applied for the last 14 patients.

Water suppression was performed using chemical shift selective (CHESS) pulses with a bandwidth of 60 Hz. For spectral editing, a 20 msec Gaussian 180° pulse was applied at 1.9 ppm for the edit-on condition and interleaved at 7.5 ppm for the edit-off condition to edit for 2-HG at 4.02 ppm in the difference spectra (64 pairs of scans). Spectroscopic parameters were: TR = 2 seconds, TE = 60 msec, receiver bandwidth = 2.5 kHz, sampling points = 2048, scan time = 4:24 minutes. Using the FLAIR images, the voxel was positioned to include the maximal amount of solid tumor tissue and to avoid hemorrhagic/cystic areas and the skull/scalp. There were 40 patients with a voxel volume of 8 cm^3, three patients with a voxel size less than 8 cm^3 (3.4 cm^3, 5.8 cm^3, 6 cm^3), and 15 patients with a voxel size greater than 8 cm^3 (10–36 cm^3).

MR Analysis

The presence of the T_2/FLAIR mismatch was independently determined in patients with grades II and III gliomas by three neuroradiologists (S.C., C.T., N.Z., with 15, 14, and 5 years of experience, respectively) blinded to IDH status using criteria previously published.[8,9]

The MRS data were analyzed by an MR physicist using the AMARES time domain fitting algorithm from jMRUI v.5.2 software.[19,20] AMARES fits the amplitude of the metabolite in the time domain, which corresponds to the area under the resonance in the frequency domain. First, edit-on, edit-off, and the subtracted spectra were visually inspected for spectral artifacts, nonconstant baseline, and subtraction errors for quality assurance. In such cases, the data were removed from further analysis. Patients with a broad FWHM of the Cho peak (>18 Hz) were excluded from the study.

The subtracted spectrum of the edited MRS acquisition was fitted using a Gaussian line shape model for the 2-HG resonance at 4.02 ppm, and double-Gaussian line shape models for glutamate and glutamine (Glx, centered at 3.75 ppm) and for gamma-

aminobutyric acid overlapped by coedited macromolecules resonances (GABA+, centered at 3.01 ppm).

Further, NAA at 2.02 ppm, Cr at 3.02 ppm, and Cho at 3.20 ppm were fitted from the edit-off spectrum using Gaussian line shapes. The amplitudes of the resonances were calculated together with their associated Cramer–Rao lower bounds (CRLBs), which represent the standard deviations, expressed as %, of the estimated amplitudes.[21,22] The signal-to-noise ratio (SNR) for 2-HG was calculated by taking the ratio of the maximum signal at 4.02 ppm minus the baseline, over the root-mean-square of the noise measured between 9 and 10 ppm. A line broadening of 1 Hz was utilized for the SNR calculation. For a semiquantitative analysis, the metabolite ratios 2-HG/NAA, 2-HG/Cho, and 2-HG/Cr were calculated. The MRS signal increases linearly with voxel volume, and the voxel volume was not the same for all patients. Thus, for each patient the 2HG, GABA+, and Glx amplitudes were normalized to what would be expected for a voxel volume of 8 cm^3. This normalization was done by multiplying the metabolite amplitude by 8 cm^3 then dividing by the actual voxel volume used for that particular patient.

Pathological Analysis

Following surgery, the diagnosis of glioma was confirmed and graded using the WHO 2016 classification by a neuropathologist (J.W., with 21 years of experience or G.J., with 25 years of experience) who were blinded to the MRS results. IDH1 mutation status was assessed by immunohistochemistry using the H09 clone (Dianova, Hamburg, Germany) generated against the R132H mutant IDH1. Bound antibody was revealed using the Leica Bond III automated immunostaining platform. Immunostaining of the tumor cell cytoplasm was considered evidence of the presence of the R132H IDH1 mutation.

Patients with grades II and III gliomas and negative immunohistochemistry for the IDH R132H mutation underwent further molecular testing for the detection of noncanonical IDH1 or IDH2 mutations using next-generation sequencing on the MassArray iPLEX platform (AgenaBioscience, SanDiego, CA). For one patient, the detection of the 2-HG peak with MRS prompted a manual review of the Neuro panel mutation definition table.

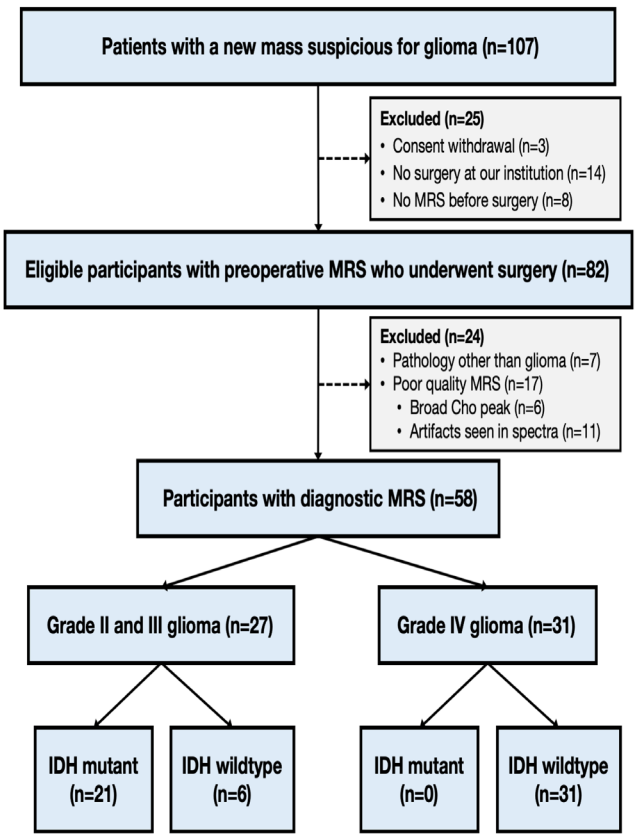

FIGURE 1: Flowchart of prospective patients who were enrolled in the study.

Statistical Analysis

Differences in 2-HG amplitude, normalized 2-HG amplitude, 2-HG/NAA, 2-HG/Cho, 2-HG/Cr, normalized Glx amplitude, and normalized GABA+ amplitude between the IDH-mutant and IDH-wildtype gliomas were assessed using a Mann–Whitney *U*-test. A Bonferroni correction was performed for multiple comparisons. $P < 0.007$ was considered statistically significant.

The areas under-the receiver operator characteristic (ROC) curve for 2-HG amplitude, normalized 2-HG amplitude, 2-HG/NAA, 2-HG/Cho, and 2-HG/Cr were calculated at multiple CRLB cutoff values (<20%, <30%, <40%, <50%, and <100%) with IDH mutation status as the outcome. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) values for the presence of the T_2/FLAIR mismatch sign were also assessed for each radiologist. Interrater reliability was measured using the intraclass coefficient correlation (ICC) using MedCalc statistical software v. 18.9 (MedCalc Software, Ostend, Belgium).

Logistic regression models based on combinations of different parameters (age, 2HG, 2HG/NAA, 2HG/Cho, 2HG/Cr) were generated to assess the accuracy of IDH-mutant prediction and ROC curves were computed. A subgroup analysis was performed for grades II and III gliomas. Comparisons between the model areas under the curve (AUCs) and those associated with individual parameters were evaluated according to the method of DeLong. $P < 0.05$ was considered statistically significant.

Results

Participants

There were 107 patients who consented and were enrolled in the study. Forty-nine of the 107 enrolled patients were excluded from the study for various reasons (eg, consent withdrawal, no surgery performed at our institution, pathologies other than glioma, inability to obtain MRS before surgery, or poor quality MRS) (Fig. 1). The median time to surgery after MRS was 9 days (range 5–13 days).

Fifty-eight patients were included in our study. The clinical and pathological information for these patients are listed in Table 1. In keeping with cohorts published elsewhere, patients harboring an IDH mutation (mean age, 42 ± 10 years) were younger than patients without the mutation (mean age, 59 ± 14 years) ($P < 0.05$). There was no statistically significant difference in the proportion of IDH mutant gliomas among males (41%) vs. females (29%)

(P = 0.36). There were six diffuse astrocytomas (five IDH-mutant, one IDH-wildtype), seven oligodendrogliomas (two grade II and five grade III), 14 anaplastic astrocytomas (nine IDH-mutant, five IDH-wildtype), and 31 glioblastomas (all IDH-wildtype). There was a significant difference in the number of patients with IDH-mutant gliomas who underwent surgical resection (100%) compared to IDH-wildtype gliomas (78%) (P < 0.05). Eight of 37 patients (22%) with IDH-wildtype gliomas had a biopsy only.

TABLE 1. Clinical and Pathological Information for Patients With IDH-Mutant vs. IDH-Wildtype Gliomas

	IDH-Mutant (n = 21)	IDH-Wildype (n = 37)
Mean age (STDEV)	42 (±10.6)	59 (±14.5)
Gender		
Female	7	17
Male	14	20
Diffuse astrocytoma (grade II)	5	1
Oligodendroglioma (grade II)	2	—
Anaplastic astrocytoma (grade III)	9	5
Anaplastic oligodendroglioma (grade III)	5	—
Glioblastoma (grade IV)	0	31
Biopsy	0	8
Resection	21	29

MRI Results

For all patients with gliomas from grades II to IV, the average choline FWHM was 11 ± 5 Hz. The average 2-HG SNR was 3.7 ± 3.0. The 2-HG amplitude, normalized 2-HG amplitude, 2-HG/Cho, and 2-HG/NAA were higher for IDH-mutant gliomas compared to IDH-wildtype gliomas (P < 0.007, Table 2). IDH-mutant gliomas had a trend of higher 2-HG/Cr (P = 0.0074) and lower normalized Glx amplitude compared to IDH-wildtype gliomas (P = 0.024, Table 2). The diagnostic accuracy of 2-HG was moderate in identifying IDH-mutant gliomas, with an AUC between 0.79 and 0.89 depending on the CRLB threshold (Table 3).

A 2-HG amplitude greater than 0 Institutional Unit (I.U.) with a CRLB less than 20% had 100% specificity (13/13; 100%, 95% confidence interval [CI]: 75–100%) and 77% sensitivity(10/13; 77%, 95% CI: 46–95%). In Fig. 2, a 2-HG amplitude of 31.8 I.U. with a CRLB of 4% is seen in a patient with an IDH-mutant glioma. When the CRLB threshold was increased to 30%, there was a decrease in specificity to 81% (13/16, 54–96%) due to three patients with glioblastomas who had a small detectable peak at 4 ppm. In Fig. 3, a 2-HG amplitude of 4.0 I.U. with a CRLB of 20.3% is visible in a patient with an IDH-wildtype glioblastoma, which could represent a false-positive MRS if a CRLB < 30% threshold is used.

For all patients with a CRLB less than 100%, an optimal cutoff value of 2-HG greater than 1.89 I.U. had a 76% specificity (28/37; 76%, 95% CI: 59–95%) and a 76%

TABLE 2. Comparison of 2-HG Amplitudes and Ratios Between IDH-Mutant and Wildtype Gliomas for All Gliomas

	IDH-Mutant (n = 21)		IDH-Wildtype (n = 37)		
Parameters	Median	Interquartile range	Median	Interquartile range	P value[b]
2-HG	3.0 I.U.[a]	1.9 to 5.4 I.U.	1.1 I.U.	0 to 1.9 I.U.	<0.007
Normalized 2-HG	2.0 I.U.	1.4 to 4.1 I.U.	1.1 I.U.	0 to 1.9 I.U.	<0.007
2-HG/Cho	0.039	0.033 to 0.052	0.016	0 to 0.036	<0.007
2-HG/Cr	0.056	0.035 to 0.096	0.023	0 to 0.050	0.0074
2-HG/NAA	0.062	0.040 to 0.106	0.018	0 to 0.050	<0.007
Normalized Glx	9.0 I.U.	6.0 to 12.5 I.U.	13.8 I.U.	7.4 to 16.7 I.U.	0.024
Normalized GABA+	8.3 I.U.	5.9 to 12.8 I.U.	12.0 I.U.	6.9 to 17.4 I.U.	0.056

Statistics are listed with their interquartile range.
[a]Institutional Units.
[b]P < 0.007 is considered statistically significant using a Bonferroni correction.

TABLE 3. Diagnostic Accuracy of 2-HG Amplitude at Different CRLB Thresholds in Differentiating Between IDH Mutant and IDH Wildtype Gliomas for All Gliomas

CRLB threshold	*N*	Area under the curve	Optimal cutoff (I.U.)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
<20%	26	0.89 (0.70–0.98)	>0	10/13 (77, 46–95)	13/13 (100, 75–100)	10/10 (100, N/A)	13/16 (81, 62–92)
<30%	31	0.85 (0.68–0.95)	>0	12/15 (80, 52–96)	13/16 (81, 54–96)	12/15 (80, 58–92)	13/16 (81, 61–92)
<40%	39	0.81 (0.65–0.92)	>2.37	13/18 (72, 47–90)	17/21 (81, 58–95)	13/17 (76, 56–89)	17/22 (77, 61–88)
<50%	47	0.78 (0.64–.0.89)	>1.95	15/20 (75, 51–91)	19/27 (70, 50–86)	15/23 (65, 50–78)	19/24 (79, 63–89)
<100%	58	0.79 (0.66–0.89)	>1.89	16/21 (76, 53–92)	28/37 (76, 59–88)	16/25 (64, 49–77)	28/33 (85, 72–92)

Statistics are listed with their 95% confidence intervals.

sensitivity (16/21; 76%, 95% CI: 53–92%). An optimal cutoff value of normalized 2-HG greater than 1.4 I.U. achieved a lower specificity (25/37; 68%, 95% CI: 50–82%) and a similar sensitivity (16/21; 76%, 95% CI: 53–92%). For the metabolite ratios evaluated, 2-HG/NAA > 0.034 had the highest sensitivity and specificity, 86% (95% CI: 64–97%) and 73% (95% CI: 56–86%), respectively (Table 4).

For the subset of patients with grades II and III gliomas, the diagnostic accuracy of 2-HG was high in identifying IDH-mutant gliomas, with the AUC between 0.89 and 0.92, depending on the CRLB threshold (Table 5). A 2-HG amplitude greater than 0 I.U. with a CRLB less than 30% had 100% specificity (4/4; 100%, 95% CI: 40–100%) and 95% sensitivity (12/15; 80%, 95% CI: 52–96%).

When all patients with a CRLB less than 100% were included, an optimal cutoff value of 2-HG > 1.24 I.U. resulted in 100% specificity (6/6; 100%, 95% CI: 54–100%) and 81% sensitivity (17/21; 81%, 95% CI: 58–95%). An optimal cutoff value of corrected 2-HG > 1.33 I.U. achieved a similar high specificity (6/6; 100%, 95% CI: 54–100%) but a lower sensitivity (16/21; 76%, 95% CI: 53–92%). Among the different metabolite ratios, 2-HG/NAA > 0.037 had the highest diagnostic accuracy, with sensitivity and specificity of 76% (95% CI: 53–92%) and 100% (95% CI: 54–100%), respectively (Table 4).

From visual assessment of conventional MR images, one patient out of 21 IDH mutant grade II or III gliomas was found by all three radiologists to have a T_2/FLAIR mismatch sign for a sensitivity of 5% (95% CI: 0–26%) (Table 6). An additional IDH-wildtype glioma had part of the tumor that was hyperintense on T_2-weighted images and hypointense on FLAIR images. This was incorrectly identified as a T_2/FLAIR mismatch by one of the three radiologists. Thus, the specificity of this sign was 100% (95% CI: 52–100%) for two radiologists and 83% (95% CI: 36–99%) for the third radiologist. There was a good interrater agreement for the T_2/FLAIR mismatch (ICC r = 0.75, 95% CI: 0.58–0.86)

For all gliomas, the combination of different MRS parameters such as 2-HG/NAA + 2HG/Cho did not improve the accuracy over 2HG or 2HG/NAA alone (Table 5). The classification model using age + 2HG/NAA had the highest diagnostic accuracy of 0.92 (95% CI: 0.81 to 0.97), a sensitivity of 91% (95% CI: 70–99%), and a specificity of 89% (95% CI: 75–97%). For grades II and III gliomas, the combination of different MRS parameters or age + 2HG/NAA did not improve the accuracy over 2HG or 2HG/NAA alone (Table 4).

Pathological Results

Among grade II and III gliomas, 18 patients had an IDH1 R132H mutation detected by immunohistochemistry. Three patients with negative immunohistochemistry had a

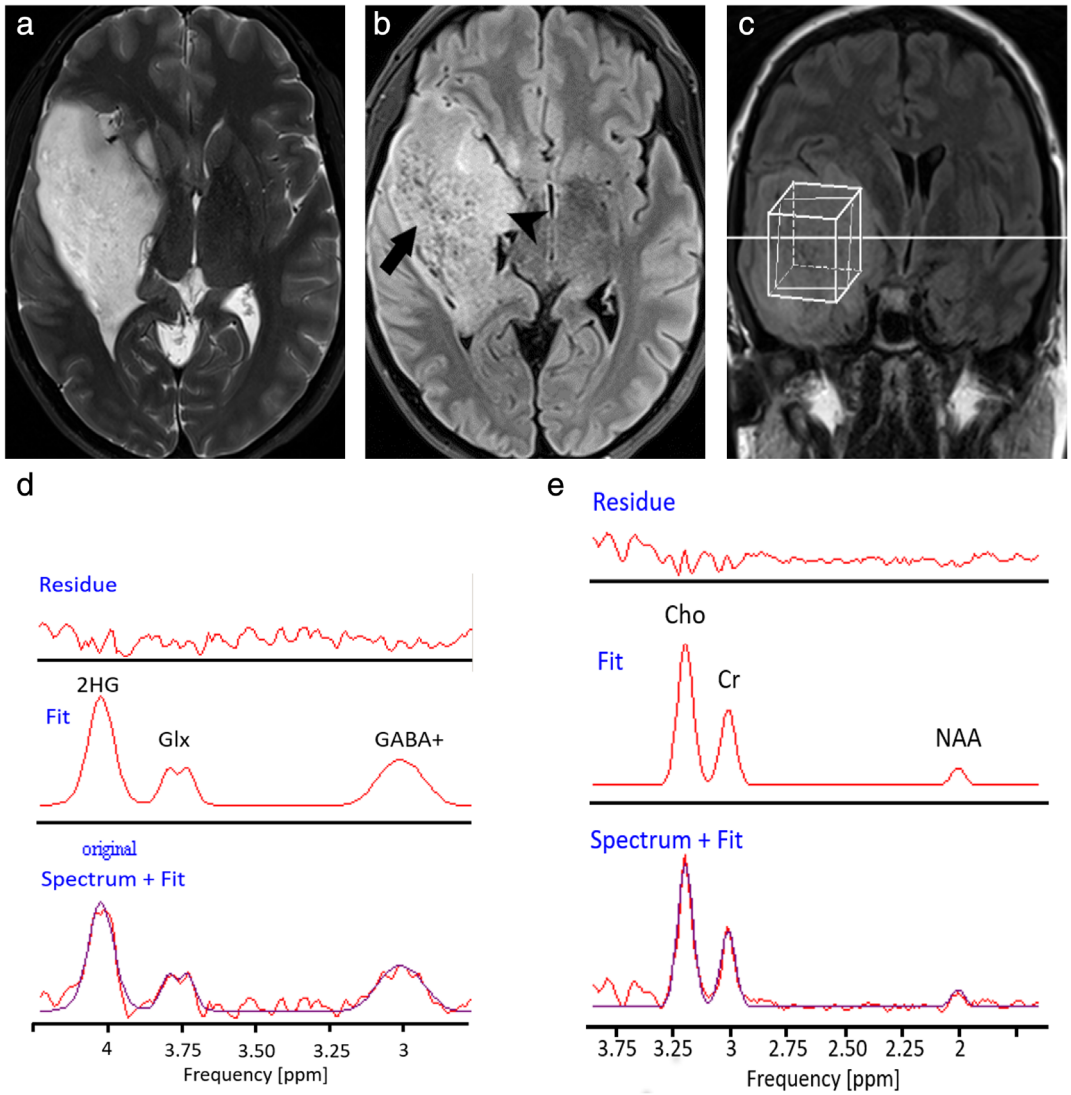

FIGURE 2: Forty-year-old patient with an IDH2 mutation. The axial T_2-weighted image (a) shows a hyperintense right temporal mass extending to the lentiform nucleus. Axial FLAIR (b) image shows loss of signal intensity only on the anterolateral part of the tumor (block arrow), while the medial part remains hyperintense (arrow). This was interpreted as negative for the T_2/FLAIR mismatch sign. Coronal FLAIR image (c) shows a $3 \times 3 \times 4$ cm^3 voxel placed in the tumor. Difference spectrum (d) shows a 2-HG peak at 4.02 ppm with a relative CRLB of 2.3%. Glutamate and glutamine (Glx) at 3.75 ppm as well as gamma-aminobutyric acid overlapped by coedited macromolecules (GABA+) at 3.01 ppm are also visible in the spectrum. Edit-off spectrum (e) shows an increase in the Cho peak and a decrease in the NAA peak relative to the Cr peak. Surgical resection revealed an anaplastic astrocytoma. Immunohistochemistry was negative for IDH1. DNA sequencing was initially negative but finally revealed an IDH2 R172S mutation.

noncanonical IDH1 or an IDH2 gene mutation by next-generation sequencing: one patient had a pontine glioma with an IDH1 R132C mutation, one patient had an anaplastic astrocytoma with an IDH2 R172K mutation, and one patient had an anaplastic astrocytoma with an IDH2 R172S mutation. The IDH2 R172S mutation was initially not described because it was omitted from the Neuro panel mutation definition table. The detection of the 2-HG peak with MRS prompted a manual review of the Neuro panel for this patient and led to the correction of this omission (Fig. 2), thereby independently validating the sensitivity of 2-HG MRS for detecting IDH mutant gliomas. All 31 patients with glioblastomas had a negative immunohistochemistry. Three out of those 31 patients had DNA sequencing that were concordant with the immunohistochemistry result.

Discussion

In this study, edited MRS achieved a high specificity and PPV in the preoperative identification of IDH-mutant gliomas in patients with grade II and III gliomas. This finding is in agreement with previous results obtained using edited MRS and is confirmed in this larger prospective cohort of patients.[14,15] The study also confirmed that the T_2/FLAIR mismatch sign has high specificity (83–100%) and low sensitivity (5%) for the prediction of the IDH mutation.[7,8] A

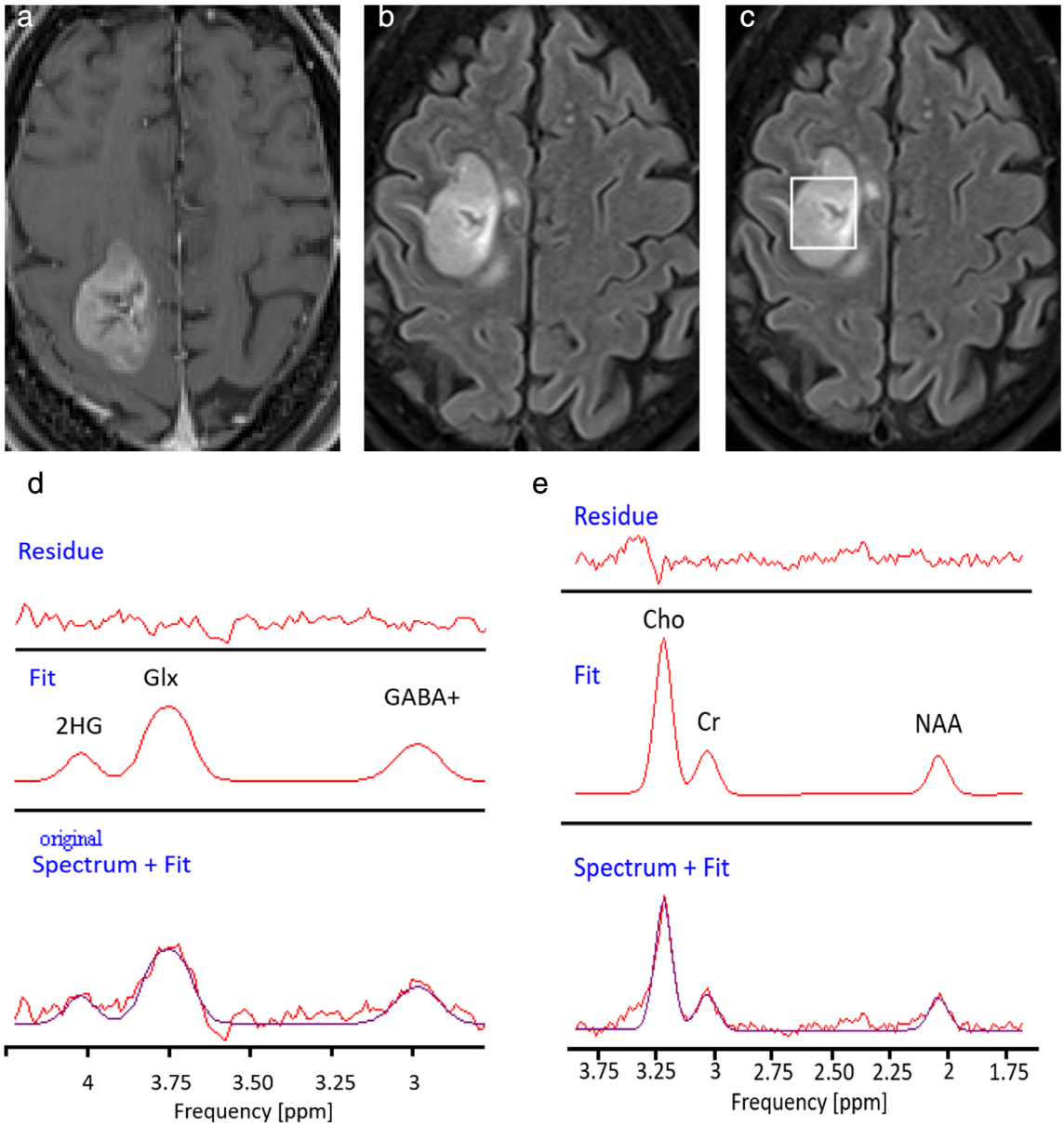

FIGURE 3: Seventy-nine-year-old patient with an IDH wildtype glioblastoma and a false-positive MRS using a CRLB <30% threshold. Axial T_1 postcontrast (a) and axial FLAIR (b) images demonstrate a mass in the right frontal lobe with a small central area of necrosis. Axial FLAIR image (c) shows a $2 \times 2 \times 2$ cm^3 voxel placed in the tumor. Difference spectrum (d) shows a 2-HG amplitude of 4.0 I.U. with a relative CRLB of 20.3% at 4.02 ppm. Edit-off spectrum (e) shows an increase in the Cho peak and a decrease in the NAA peak relative to the Cr peak. Immunohistochemistry was negative for IDH1.

small 2-HG peak was found in a few patients with glioblastomas, which has not been documented with previous studies using edited MRS because only a few patients with glioblastomas were included.[14,15] Because of the false-positive cases among glioblastomas, the specificity of edited MRS was lower in the detection of IDH mutation for all glioma patients compared to the subset of patients with grade II and III gliomas. There was no increase in diagnostic accuracy from the use of multiple different MRS metabolites over 2-HG or 2-HG/NAA alone.

False-positive cases of IDH mutations have previously been reported with other MRS methods but not with edited MRS. Both short- and long-echo PRESS sequences detect the 2-HG peak at 2.25 ppm, which can be overlapped by the presence of increased glutamate–glutamine levels (methylene groups at 2.1–2.4 ppm) and lipids (2.0–2.9 ppm). This is not a likely cause of false positivity in our study, since edited MRS detects 2-HG peak at 4.02 ppm. False-positive 2-HG cases have also been linked to glioblastomas with necrosis and high lactate.[23,24]

The small peak seen at 4.02 ppm in a few patients with GBM could be due to noise, an overlap with lactate, or a low 2-HG concentration; however, without ex vivo tissue measurement of 2-HG we cannot determine which is the correct

TABLE 4. Diagnostic Accuracy of 2-HG Concentration Ratios and Combined Parameters in Differentiating Between IDH-Mutant and IDH-Wildtype Gliomas for All Gliomas (n = 58) and for Grade II and III Gliomas (n = 27) Using a CRLB Threshold<100%

Parameter	Area under the curve	Optimal cutoff (I.U.)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
For all gliomas (n = 58)						
2-HG/NAA	0.80 (0.67 to 0.89)	>0.034	18/21 (86, 64–97)	27/37 (73, 56–86)	18/28 (64, 51–76)	27/30 (90, 76–96)
2-HG/Cho	0.74 (0.61 to 0.85)	>0.024	18/21 (86, 64–97)	25/37 (68, 50–82)	18/30 (60, 48–71)	25/28 (89, 74–96)
2-HG/Cr	0.71 (0.58 to 0.82)	>0.033	17/21 (81, 58–95)	23/37 (62, 45–78)	17/40 (43, 35–51)	14/18 (78, 57–90)
2-HG/Cho + 2-HG/Cr + 2-HG/NAA	0.81 (0.69 to 0.90)	>0.340	16/21 (76, 53–92)	31/37 (84, 68–94)	16/22 (72, 55–85)	31/36 (88, 74–93)
2-HG/Cho + 2-HG/NAA	0.81 (0.69 to 0.90)	>0.340	16/21 (76, 53–92)	31/37 (84, 68–94)	16/22 (72, 55–85)	31/36 (88, 74–93)
2-HG/NAA + Age	0.92 (0.81 to 0.97)	>0.370	19/21 (91, 70–99)	33/37 (89, 75–97)	19/23 (82, 65–92)	33/35 (94, 81–98)
For grade II and III gliomas (n = 27)						
2-HG/NAA	0.89 (0.71 to 0.98)	>0.037	16/21 (76, 53–92)	6/6 (100, 54–100)	16/16 (100, N/A)	6/11 (55, 36–72)
2-HG/Cho	0.82 (0.62 to 0.94)	>0.027	17/21 (81, 58–95)	5/6 (83, 36–100)	17/18 (94, 74–99)	5/9 (56, 33–76)
2-HG/Cr	0.87 (0.69 to 0.97)	>0.023	18/21 (86, 64–97)	5/6 (83, 36–100)	18/19 (95, 75–99)	5/8 (63, 36–83)
2-HG/Cho + 2-HG/Cr + 2-HG/NAA	0.96 (0.80 to 1)	>0.500	18/21 (86, 64–97)	6/6 (100 (48–100)	18/18 (100, NA)	6/9 (67, 41–85)
2-HG/Cho + 2-HG/Cr	0.96 (0.80 to 1)	>0.500	18/21 (86, 64–97)	6/6 (100, (48–100)	18/18 (100, NA)	6/9 (67, 41–85)
Age + 2-HG/NAA	0.87 (0.69 to 0.97)	>0.760	17/21 (81, 58–94)	6/6 (100, 54–100)	17/17 (100, NA)	6/10(60, 38–78)

Statistics are listed with their 95% confidence intervals.

TABLE 5. Diagnostic Accuracy of 2-HG Amplitude at Different CRLB Thresholds in Differentiating Between IDH Mutant and IDH Wildtype Gliomas for Grade II and III Gliomas

CRLB threshold	*n*	Area under the curve	Optimal cutoff (I.U.)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
<20%	17	0.86 (0.64–0.99)	>0	10/13 (77, 46–95)	4/4 (100, 40–100)	10/10 (100, N/A)	4/7 (57, 33–79)
<30%	19	0.90 (0.68–0.99)	>0	12/15 (80, 52–96)	4/4 (100, 40–100)	12/12 (100, N/A)	4/7 (57, 33–79)
<40%	22	0.92 (0.72–0.99)	>0	15/18 (83, 59–96)	4/4 (100, 40–100)	15/15 (100, N/A)	4/7 (57, 33–79)
<50%	25	0.91 (0.73–0.99)	>0.97	17/20 (85, 62–97)	5/5 (100, 48–100)	17/17 (100, NA)	5/8 (63, 37–83)
<100%	27	0.90 (0.72–0.98)	>1.24	17/21 (81, 58–95)	6/6 (100, 54–100)	17/17 (100, NA)	6/10 (60, (38–78)

Statistics are listed with their 95% confidence intervals.

interpretation for a specific patient. The methine proton of lactate at 4.11 ppm should ideally be canceled out on the difference spectrum. But, if a small frequency shift occurs, the methyl group of lactate at 1.3 ppm might be coedited with the Gaussian editing pulse centered at 1.9 ppm so that a high lactate content might lead to a visible peak at 4.11 ppm in the difference spectrum. Branzoli et al reported one patient who had a wildtype glioma with a 2-HG concentration of 2.9 nmol/mg.[15] Biochemical studies have reported that 2-HG can be produced by the wildtype IDH1 enzyme or by lactate dehydrogenase under hypoxic conditions.[25,26]

Edited MRS offers some distinct advantages over other MRS techniques in the detection of 2-HG. For example, at a similar CRLB threshold the false-positive rate with edited MRS appears lower compared to that reported by other MRS techniques, such as short or long TE PRESS, which have a false-positive rate up to 21%.[23,24,27] The second advantage is that the 2-HG peak can be visualized directly at 4.02 ppm on the difference spectrum without overlap from other metabolites.

However, there are some drawbacks to this methodology that deserve consideration. First, the acquisition time for the edited MRS sequence takes twice as long as a regular PRESS sequence, since two acquisitions (one with the editing pulse at 1.9 ppm [edit-on] and one with the editing pulse at 7.5 ppm [edit-off]) must be performed to obtain a difference spectrum. Thus, edited MRS is more susceptible to motion artifacts. However, retrospective frequency and phase correction can be performed to improve the quality of the MR spectrum.[28] Second, edited MRS has a lower SNR than regular PRESS. It is able to detect 2-HG in tumors ≥8 cm^3 but we could not evaluate its diagnostic accuracy for smaller tumors (<8 cm^3) due to the small number of patients with a small tumor size in our study. Using a PRESS sequence for detection of 2-HG, de la Fuente et al[13] reported a sensitivity of 47% for tumors between 3.4 and 8 cm^3 compared to 91% for tumors greater than 8 cm^3. In theory, the SNR can be increased by a factor of $\sqrt{2}$ by doubling the number of acquisitions (128 instead of 64), but at the expense of doubling scan duration, which would increase the likelihood of motion artifacts. Finally, the MEGA-PRESS sequence is still not available for clinical purposes on many commercial scanners, although it is readily available as a research sequence for the detection of gamma-aminobutyric acid (GABA) in the brain.

Limitations

The lack of DNA sequencing for IDH1 and IDH2 mutations in 28 patients with GBM is not performed routinely for clinical care at our institution. Ten of 28 patients with GBM were younger than 55, which is the age cutoff recommended for DNA sequencing.[29] If one of those patients had a noncanonical IDH1 or 2 mutation, this could have affected the diagnostic accuracy of MRS. Furthermore, we did not measure the signal of unsuppressed water and were not able to

TABLE 6. Diagnostic Accuracy of the T_2/FLAIR Mismatch Sign in the Detection of IDH Mutation for Grade II and III Gliomas (n = 27)

Radiologists	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
#1	1/21 (5, 0–26)	5/6 (83, 36–99)	1/2 (50, 3–97)	5/25 (20, 8–41)
#2	1/21 (5, 0–26)	6/6 (100, 52–100)	1/1 (100, 5–100)	6/26 (23, 10–44)
#3	1/21 (5, 0–26)	6/6 (100, 52–100)	1/1 (100, 5–100)	6/26 (23, 10–44)

Statistics are listed with their 95% confidence intervals.

perform absolute quantification of 2-HG. Nevertheless, relative metabolite ratios such as 2-HG/Cr or 2-HG/NAA can be readily calculated from edited MRS and can be used to identify IDH mutant gliomas. Finally, the small sample size of our study limits the power of the multivariate analysis to determine the most accurate combination of relevant metabolites in the classification of IDH mutant gliomas.

Conclusion

We have found that spectral edited MRS appears to have a high specificity in the prediction of IDH mutation in patients with grade II and III gliomas, but lower specificity in patients with glioblastomas. To avoid false-positive cases in patients with glioblastomas, a strict CRLB threshold <20% might be recommended.

Acknowledgments

The MEGA-PRESS sequence was developed by Edward J. Auerbach, Ph.D., and Małgorzata Marjańska, Ph.D., and provided by the University of Minnesota under a C2P agreement.

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