Introduction
Somatic mutations in the enzyme isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) have been identified in astrocytic and oligodendrogial tumors of WHO grades II and III, and in secondary glioblastoma (GBM) [1]. The IDH1 and IDH2 mutations are associated with elevated levels of 2-hydroxylutarate (2HG) and may serve as a clinical biomarker for disease stratification and prognosis [2]. We have been studying metabolism of glucose in the citric acid cycle of tumors by infusing [U-13C]glucose and taking biopsies of tissue at the time of surgical resection for later analysis. These samples can also be used to investigate the presence of 2HG by 1H-NMR methods. The aim of this study was to measure the concentration of 2HG by 1H-NMR in the extracts.

Methods
Tissue samples from 22 patients with gliomas were examined: 7 IDH1/2 mutant (e.g., 3 WHO grade II and 4 WHO grade III) and 15 wild type tumors (e.g., 1 WHO grade I, 2 WHO grade II, 2 WHO grade III, and 10 WHO grade VI). These samples were taken from patients receiving an infusion of [U-13C]glucose. The samples ranged from 200 to 700 (mean ± SD, 490 ± 210) mg wet weight. Percholric acid extracts of the tissues were redissoled in 0.2 mL of deuterium oxide. 1H-NMR spectroscopy was performed on a Varian 600MHz spectrometer using 3 mm broadband NMR probe. The spectral parameters were as follows: 90° pulse angle, 7225 Hz sweep width, 32K complex data points, relaxation delay 0.05 s and total acquisition time of 23 min. The absolute metabolite concentrations were calculated using equation: [C]i = (N12pNj) × (S/S0) × (mol/Msample), where [C]i is the concentration of the metabolite (μmol/g), S is the amplitude of the metabolite and S0 is the signal amplitude of 3-(Trimethylsilyl)propionic acid (TSP), mol is the number of moles the TSP and Msample is the weight of the sample. The terms N1 and N2 represent the number of 1H nuclei contributing to the resonance of metabolites i (i.e., 2HG, Glu, Gln, and GABA) and TSP.

Results
Figures 1 and 2 demonstrate representative high resolution one-dimensional 1H-NMR on glioma samples with IDH1/2 mutated and wild type tumors. 2HG signal was detected in the IDH mutated tumors but not wild type tumors. A 2HG molecule has two methylene groups (δCH2) and a methine group (δCH) that give NMR signals at 4.03, 2.26, 1.29, and 1.94 ppm. Three of four multiplets of 2HG were observed (Figure 1). On the 1H-NMR spectra, the 2HG resonances were partially overlapped with those of GABA, Glu, Gln, and NAA. In this study 2HG (δCH2 at 2.26 ppm), Glu (δCH2 at 2.36 ppm), Gln (δCH at 2.46 ppm), and GABA (δCH2 at 2.50 ppm) metabolites were measured by fitting a Voigt (Gauss and Lorentz) function. 1H-NMR result was positive for 2HG in all tumors that contained IDH1/2 mutation but negative in all tumors with wild type . The measured 2HG levels ranged from 0.55 to 3.51 (mean ± SD, 2.00 ± 1.02) μmol/g. COSY spectroscopy and authentic solutions were used to confirm assignments.

Discussion
This study supports the use of 2HG as a biomarker of IDH1/2 mutation status in glioma [1, 3]. With the chemical shift dispersion that is available for analysis of tissue extracts, signals from Gln, Glu and GABA were easily resolved. The levels of 2HG in this work had a range of 0.55 – 3.51 μmol/g from seven glioma samples with IDH1/2 mutation (3 WHO grade II and 4 WHO grade III), which is consistent with the finding of previous in vivo 1H-MRS study (e.g., 1.7 – 8.9 mM, Choi et al. [4]). Dang et al. [2] reported that the range of 2HG by mass spectroscopy was from 5 to 35 μmol/g. In addition, Glu levels were found to be lower in IDH1/2 mutant tumors compared to IDH1/2 wild type tumors (0.80 vs. 1.98 μmol/g, P < 0.0001). This result reflects that IDH mutations cause a decrease in Glu and/or α-ketoglutarate production and an increase in 2HG. Two-dimensional 1H-NMR spectroscopy (e.g., COSY) may help detect and assign the resonances difficult to observe in the one-dimensional 1H-NMR spectra (Figure 3).

References