Magnetic Resonance Imaging (MRI) and Spectroscopy in Succinic Semialdehyde Dehydrogenase Deficiency

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Abstract

Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal recessive disorder of γ-aminobutyric acid (GABA) degradation, resulting in elevations of brain GABA and γ-hydroxybutyric acid (GHB). Previous magnetic resonance (MR) spectroscopy studies have shown increased levels of Glx in SSADH deficiency patients. Here in this work, we measure brain GABA in a large cohort of SSADH deficiency patients using advanced MR spectroscopy techniques that allow separation of GABA from overlapping metabolite peaks. We observed significant increases in GABA concentrations in SSADH deficiency patients for all 3 brain regions that were evaluated. Although GABA levels were higher in all 3 regions, each region had different patterns in terms of GABA changes with respect to age. We also report results from structural magnetic resonance imaging (MRI) of the same cohort compared with age-matched controls. We consistently observed signal hyperintensities in globus pallidus and cerebellar dentate nucleus.

Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal-recessive disorder of GABA metabolism.[1] Currently approximately 400 patients have been identified around the world, with about 85% younger than 18 years. SSADH in combination with GABA transaminase converts GABA to succinate. The absence of SSADH leads to GABA buildup and conversion of GABA to γ-hydroxybutyric acid (GHB), leading to central nervous system GABA and γ-hydroxy butyrate (GHB) accumulation. Patients with SSADH deficiency present with motor delay, cognitive and language delay, hypotonia, ataxia, behavioral problems, hyperkinesis, and seizures. The key biochemical finding is elevated GHB in urine and plasma of affected patients. SSADH deficiency does not show widespread metabolite deterioration during systemic challenges such as febrile illnesses, and the clinical phenotype is heterogeneous even within families, thus rendering the diagnosis difficult.

Neuroimaging techniques such as magnetic resonance imaging (MRI) and electrophysiologic techniques such as electroencephalography (EEG) have the potential to improve accuracy of diagnosis and monitoring of progression in SSADH deficiency patients. EEG abnormalities such as generalized and focal epileptiform discharges, photosensitivity, and background slowing have been reported in the literature.[2] Neuroimaging has demonstrated increased T2-weighted MRI signal affecting the globus pallidus, cerebellar dentate nucleus, and subthalamic nucleus with variable cerebral/cerebellar atrophy; in one report, diffusion restriction and abnormal perfusion has also been detected in the globus pallidus.[3-6] Magnetic resonance spectroscopy (MRS) has shown elevated glutamine/glutamate (Glx) peaks.[7] However, most of these findings are based on isolated case reports.

Here in this work, we report results from an imaging study where 18 SSADH deficiency patients and 13 age-matched

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Table 1. Summary of Quantitative (Left) and Qualitative (Right) Metrics Used to Evaluate Structural MRI Data.

Ventricle size (frontal occipital horn ratio, atrial dimension)	Cerebral gray/white matter volume
Thickness of periatrial white matter	Cerebellar atrophy
Gray matter measures (eg, coronal caudate thickness at the anterior commissure)	Myelination score (adapted from Plecko et al[16])
Cerebral atrophy (subarachnoid spaces, sulci)	Dysmyelination
Cerebellar volume measures (transverse diameter, width of primary vermian fissure)	Parenchymal injury
Midpons anteroposterior dimension[17]	Metabolic signal abnormality (globus pallidus, putamen, thalamus, dentate, subthalamic nucleus)
Corpus callosum measures (genu/ splenium thickness, anteroposterior dimension)[18]	Malformations (eg, vermian hypoplasia)
Ellipsoid estimate for volume of globus pallidus and dentate signal abnormality	Miscellaneous (eg, arachnoid cysts, olfactory hypoplasia)

controls were evaluated with a 1-hour long MRI and MRS protocol. We report our findings on structural MR imaging, particularly by evaluating the globus pallidus, subthalamic nucleus, and dentate nucleus. We also report our findings from single-voxel spectroscopy as well as a novel approach using j-edited Mescher-Garwood Point Resolved Spectroscopy (MEGAPRESS) sequence that is capable of direct measurement of GABA.

Materials and Methods

Subjects

This study was a part of the Natural History Study of Patients With Succinic Semialdehyde Dehydrogenase (SSADH) Deficiency conducted in 4 academic institutions: Washington State University, Boston Children's Hospital, University of South Florida, and University Children's Hospital Heidelberg. A total of 25 patients were recruited at the Boston Children's Hospital. These patients underwent a comprehensive evaluation including history/physical, neuropsychological testing, EEG, TMS, MRI, and bio-specimen collection over the course of 2 days. The patients are also followed with questionnaires and surveys sent out every 6 months, and bio-specimen collection every year. Age-matched controls were also recruited for the study and went through the same tests for comparison.

MRI

All experiments were performed on a whole-body 3-tesla MRI scanner (Siemens Skyra) equipped with a 64-channel phased array head coil. The study was approved by the local IRB, and participants and families gave written informed consent for participation. Subjects were not sedated but were offered a movie during the scan through MR-compatible goggles, to reduce the amount of motion. Among 25 subjects, 18 of them were able to complete the MRI study where at least 1 high-quality structural image was acquired.

Structural MRI Protocol and Analysis

Protocol. The MRI protocol consisted of

1. T1-weighted MPRAGE sequence; 1 mm isotropic resolution;
2. T2-weighted fast spin echo image acquired in axial and coronal planes; 0.4 mm in plane resolution with 2-mm slice thickness.
3. Fat suppressed 3D fluid-attenuated inversion recovery sequence in the sagittal plane; 0.9-mm isotropic resolution.

Analysis. Structural images were reviewed by a pediatric neuroradiologist (E.Y.) with more than 10 years' experience. Table 1 summarizes the evaluation metrics and criteria. The images were also evaluated with respect to quality of images and amount of motion in the images (minimal to none, mild, moderate-severe).

MR Spectroscopy Protocol and Analysis

We used a GABA-specific MEGA PRESS sequence[8] acquired in a single voxel with size 27 cc (30 mm × 30 mm × 30 mm). The sequence runs 2 acquisitions, one with an editing pulse placed at 1.9 ppm, which allows for the selective refocusing of the GABA multiplet at 3.0 ppm (called edit ON), and the other the inversion placed elsewhere (called edit OFF), which allows for GABA J-evolution. Creatine signal is suppressed and outer lines of GABA triplet (shown as a multiplet) at 3 ppm is detected when subtracting these 2 spectra (called the difference spectrum). Sequence parameters were repetition time (TR) = 1500 ms, echo time (TE) = 68 ms, and 128 averages, bandwidth = 1200 Hz. Total scan time for each voxel was 6 minutes and 30 seconds.

We have collected data from 3 different regions in the brain of each patient: basal ganglia, posterior cingulate gyrus, and occipital lobe.

T1- and T2-weighted images were used for voxel placement. Sample voxel placement in these 3 regions and corresponding spectra are shown in Figure 1.

Spectroscopy analysis. Spectroscopy data was saved and processed offline using the LCModel[9] software (version 6.3). We have used the difference spectrum from advanced spectroscopy sequence for GABA analysis using the simulated MEGAPRESS basis set provided with the software. In order to remove potential bias due to neuronal loss, we use the ratio of GABA/NAA. We have also used the OFF spectrum for PRESS analysis to calculate measurements of *N*-acetylaspartate (NAA), Creatine (Cr) and Choline (Cho).

Spectroscopy data quality control. As this was a study on a group of patients who had difficulty staying still during long scan times, we performed an analysis on spectroscopy data quality. We used 3 metrics: (1) full width at half maximum (FWHM) as a metric of linewidth; (2) signal to noise ratio (SNR), defined as the ratio of the maximum point of the spectrum to twice the residual error, as a metric of noise level; and (3) estimated standard deviations (%SD) or Cramer-Rao Lower Bound, expressed as percentage of the estimated concentrations as a metric of fit quality. We set threshold suggested in literature; less than 0.08 ppm for FWHM, greater than 10 for SNR and less than 20% for %SD.

Statistical analysis. Structural MRI and MRI spectroscopy variables were tested for significance of differences between SSADH deficiency and control subjects using a 2-tailed *t* test for continuous variables and a chi-squared test for categorial variables. Ellipsoid estimates for the
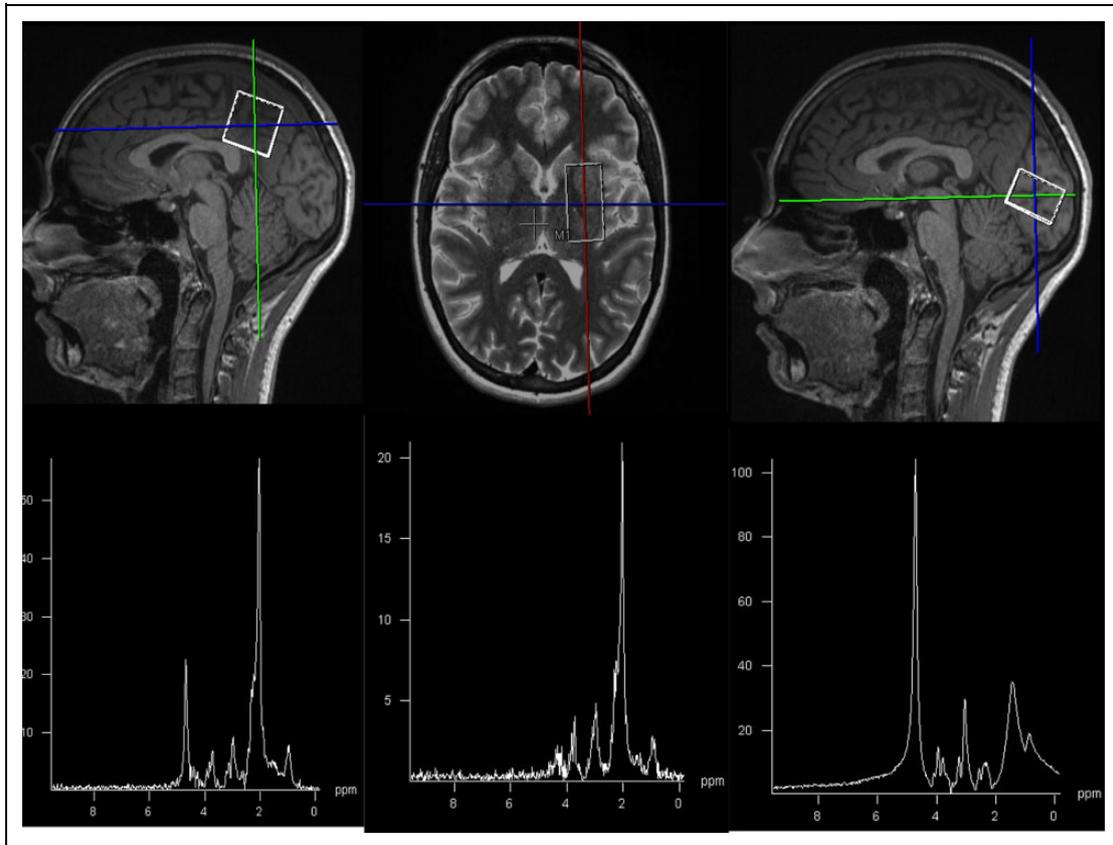
Figure 1. Sample images showing voxel placement and corresponding spectra in posterior cingulate gyrus (left), basal ganglia (middle), and occipital lobe (right).
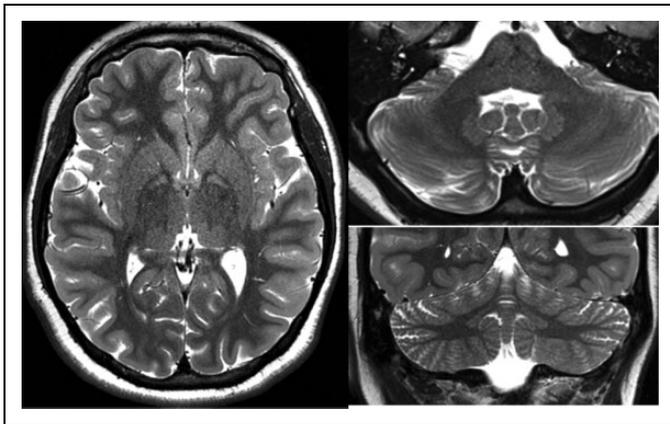

Figure 2. Representative T2-weighted imaging from SSADH patients, highlighting the hyperintense signal in globus pallidus (left) and dentate nuclei (right).

volume of dentate signal abnormality were modeled against subject age using linear regression.

Results

Structural MRI recapitulated the typical imaging findings reported for SSADH deficiency. Specifically, 14 of 16 scorable

SSADH deficiency subjects demonstrated globus pallidus T2 prolongation, and 17 of 17 scorable SSADH deficiency subjects demonstrated dentate T2 prolongation (Figure 2). Though reported in the literature, subthalamic and white matter signal abnormalities were not convincingly detected in our SSADH deficiency subjects. None of these regions had signal abnormalities in any of the control subjects. Although caution is warranted, we also detected a mild trend toward decreased dentate signal with SSADH deficiency subject age using an ellipsoid estimate for volume of dentate T2 hyperintensity ($P = .03$). Caudate nucleus ($P = .02$) and periatrial white matter ($P < .0001$) thickness were also statistically decreased in our SSADH cohort compared with normal controls. Cerebellar atrophy and thinning of the brainstem (pons) were uniquely seen in our SSADH patients (3 subjects and 2 subjects respectively) but did not meet statistical significance.

Figures 3 and 4 show the results from PRESS analysis. The NAA/Cr ratio was significantly lower ($P < .05$) in SSADHD patients in the posterior cingulate gyrus, whereas the NAA/Cr ratio was not significantly different in the basal ganglia and occipital lobe. Cho/Cr ratio was not significantly different in any of the targeted areas between SSADHD patients and control subjects.

Figure 5 summarizes results from the LCModel analysis from the edited MEGAPRESS sequence. In all 3 regions where
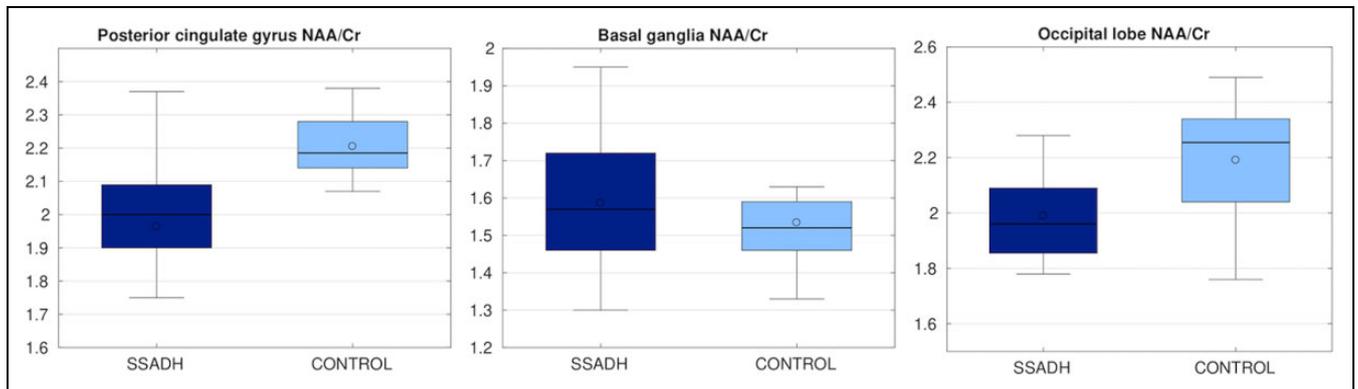
Figure 3. NAA/Cr ratio in 3 different regions, compared between SSADH patients and control subjects. The control group had a significantly higher NAA/Cr ratio.
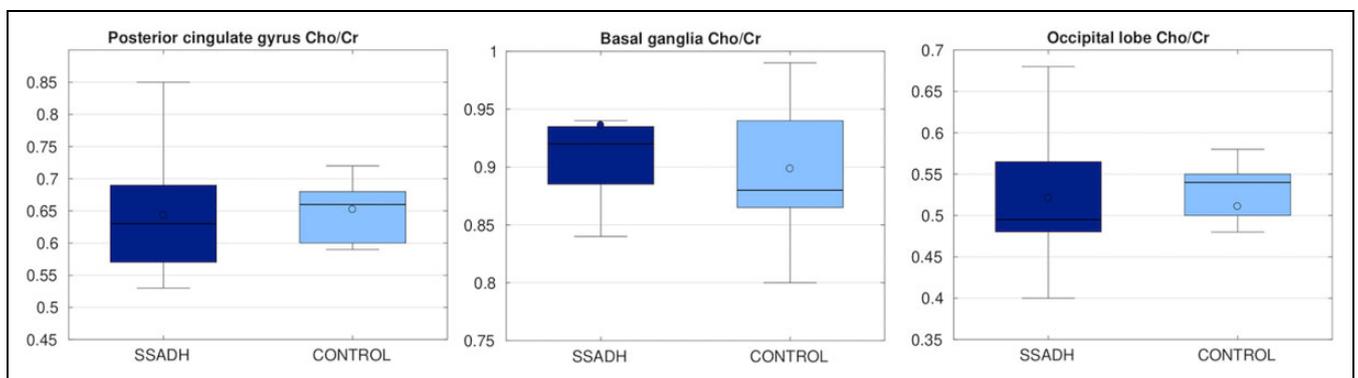

Figure 4. Cho/Cr ratio in 3 different regions, compared between SSADH patients and control subjects. There was no significant difference between 2 groups.
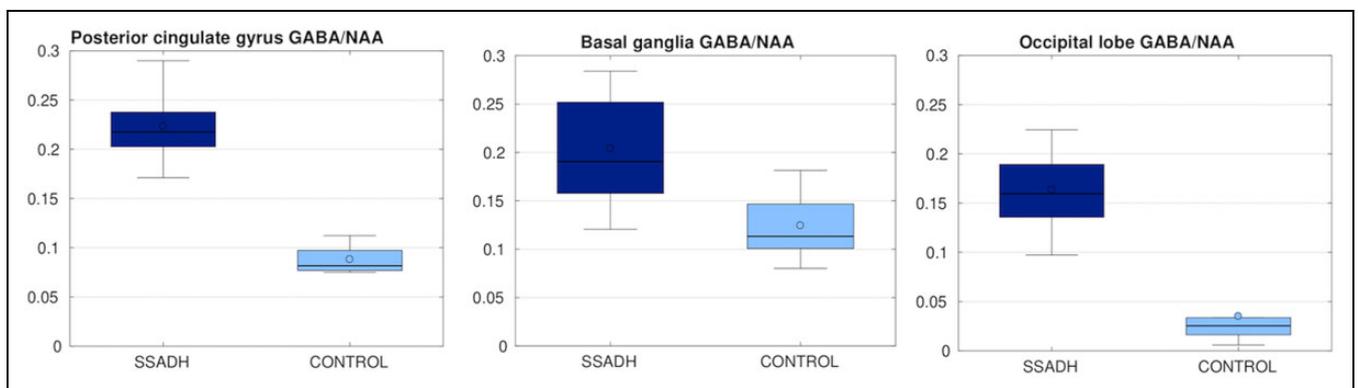

Figure 5. GABA/NAA ratio in 3 different regions, compared between SSADH patients and control subjects. In all 3 regions, GABA/NAA was significantly higher in SSADH patients.

GABA was measured, SSADH deficiency patients had significantly higher ($P < .05$) GABA/NAA ratios. In the posterior cingulate gyrus, the 2 groups were completely separated, and on average the GABA/NAA ratio was nearly 3 times larger in SSADH deficiency patients. The ratio of GABA/NAA in the occipital lobe of the controls was lower than that in the posterior cingulate gyrus and basal ganglia, but again the SSADH deficiency patients had nearly 3 times more GABA in the occipital lobe. Although the GABA/NAA was significantly higher in basal ganglia, the difference was lower compared with the other regions.

Figures 6, 7, and 8 show the change of GABA/NAA with respect to the age of subjects in the 3 regions where GABA was measured. In the posterior cingulate gyrus, the controls had a stable GABA/NAA ratio, whereas it showed a slight decline with respect to age in SSADH deficiency patients. The change in GABA/NAA with respect to age was more pronounced in the basal ganglia. For younger subjects, there was a clear
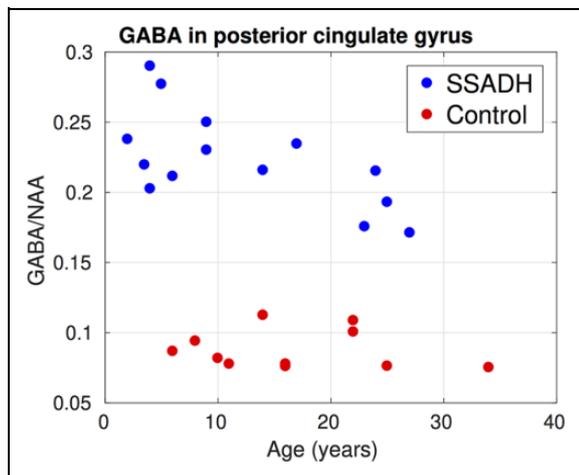
Figure 6. Change of GABA/NAA ratio in the posterior cingulate gyrus with respect to subject age. Two groups were completely separated. In the controls, the GABA/NAA ratio was mostly constant over age, whereas in SSADH patients, there was a small decline with respect to age.
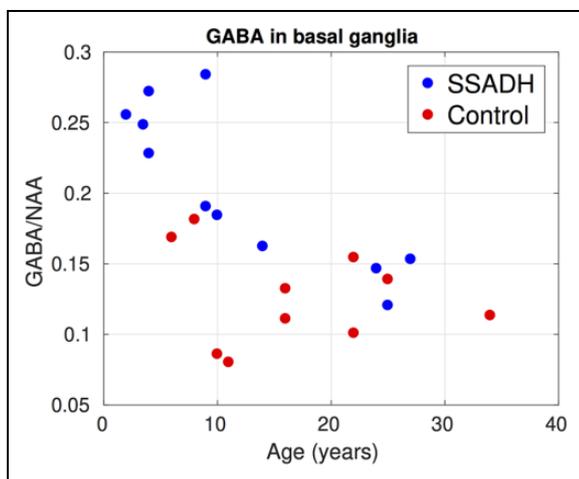

Figure 7. Change of GABA/NAA ratio in the basal ganglia with respect to subject age. Although the GABA/NAA ratio was higher in younger SSADH subjects, in the older subjects, there was no significant difference between the 2 groups.

separation between SSADH deficiency patients and control subjects, whereas the groups were not separable for older subjects. There was no clear pattern observed in GABA/NAA in the occipital lobe with respect to age.

Discussion

We observed significant increases in GABA concentrations in SSADH deficiency patients for all 3 brain regions that were evaluated. Although GABA levels were higher in all 3 regions, each region had different patterns in terms of GABA changes with respect to age. Most strikingly, we observed that GABA amounts in basal ganglia reduce with age and similar GABA levels in basal ganglia were observed in older children
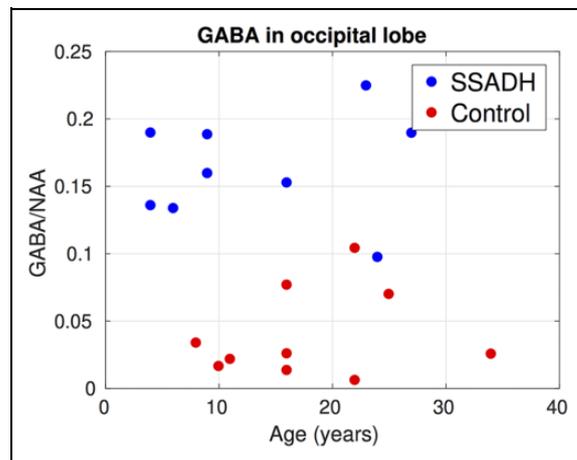

Figure 8. Change of GABA/NAA ratio in the occipital lobe with respect to subject age. The GABA/NAA ratio was significantly higher in SSADH deficiency patients.

compared to control subjects. Similar age-related correlations with plasma GABA concentrations have been noted in SSADH deficiency.[10,11] In the 2 other regions GABA levels were higher for all age groups. It should be noted that these GABA changes were not dependent on the NAA losses observed but that the GABA production per neuron was impacted by SSADH deficiency.

We did not observe any significant differences in Cho/Cr ratio between SSADH deficiency patients and control group. The NAA/Cr ratio in posterior cingulate gyrus was significantly lower in SSADHD patients compared with controls. A previous study on postmortem tissue analysis[12] observed decreased creatine levels in SSADH deficiency patients but the decrease was not statistically significant. Although NAA/Cr ratio was slightly lower in the occipital lobe of SSADH deficiency patients, this result did not achieve statistical significance.

As this was a group of patients who manifest developmental delay and intellectual disability, subject motion during the MRI acquisition was an important factor to consider. The MRI scanner is a confined and noisy environment and subjects were required to stay motionless during each imaging sequence (typically 5 minutes). As a result, young children and patients of all ages with developmental delay typically undergo sedation for an MRI examination. However, the MRI and MR spectroscopy were performed solely for research and therefore sedation was not used. Additionally, it has been previously shown that sedatives such as propofol may affect the GABA measurements.[13] Among 18 SSADH deficiency patients, 5 of them had mild motion artifacts and 6 of them had moderate to severe motion artifacts. By comparison, only 1 control had mild motion artifacts and another one had moderate motion artifacts (P value under χ^2 distribution of .04). Motion was also an important cofounder in spectroscopy data, as the MEGA-PRESS sequence requires the subject to stay still during the on and off acquisitions. Therefore, only 14 subjects met the quality control metrics for inclusion in this study.

Previous neuroimaging studies of SSADH deficiency patients have shown increased T2-weighted MRI signal affecting the globus pallidus, cerebellar dentate nucleus, and subthalamic nucleus with variable cerebral and cerebellar atrophy. In our uniformly ascertained cohort, we consistently observed signal hyperintensities in globus pallidus and cerebellar dentate nucleus, but not in the subthalamic nucleus. Motion artifact could potentially explain this lack of subthalamic signal though the subthalamic site is less consistently reported in the literature, suggesting that it may be a less common imaging manifestation of SSADH deficiency. Although not reaching statistical significance, 3 of 18 SSADH deficiency patients and no control patients demonstrated cerebellar atrophy.

The symmetric, mass neutral, and homogeneous T2 hyperintensity of the globus pallidi and dentate nuclei in SSADH deficiency patients is not pathognomonic. Nonetheless, this pattern suggests a limited number of toxic/metabolic conditions.[14,15] For example, globus pallidus signal abnormality is common in organic acidoses, and some of the diagnoses in this category are also commonly associated with dentate signal abnormality (eg, L-2-hydroxyglutaric aciduria, glutaric acidemia type 1). However, disorders in this category often have additional signal abnormalities which are not reported in SSADH deficiency: L-2-hydroxyglutaric aciduria is associated with subcortical T2 prolongation, and glutaric acidemia type 1 is associated with broader signal abnormality in the central tegmental tracts and thalami as well as opercular widening. Like mitochondrial disorders that can have myriad imaging manifestations including pallidal/dentate signal abnormality, organic acidemias also differ from SSADH deficiency by their propensity for metabolic crises and periods of developmental regression. Infantile gangliosidoses may also involve the basal ganglia and have cerebellar atrophy. However, they are frequently seen in association with broad disturbances of myelination and other abnormalities unique to this class of disorders (eg, cherry red macule, low T2 signal of the thalami). Gangliosidosis-related basal ganglia signal abnormality is also typically not confined to the globus pallidus. Guanidinoacetate *N*-methyltransferase (GAMT) deficiency is associated with globus pallidus abnormalities and can have similar clinical symptomatology to SSADH deficiency (ie, seizures, developmental delay, and behavioral problems). However, GAMT deficiency does not have dentate signal abnormality and has a unique appearance on MR spectroscopy, namely, depressed creatine levels. Toxic causes of dentate and globus pallidus signal abnormalities include vigabatrin exposure and kernicterus. However, these entities can be distinguished by their typically broader, heterogeneous pattern of signal abnormality (eg, thalami and central tegmental tracts with vigabatrin and hippocampus in kernicterus) as well as medication and birth history. Therefore, the finding of globus pallidus and dentate signal abnormality should prompt consideration of SSADH deficiency, especially in the setting of clinical data arguing against disorders with partly overlapping imaging features.

Although there is not a confirmed correlation between phenotype severity with imaging in SSADH deficiency, reports of choreoathetosis, especially in acute infantile presentations,[3,6] and ataxia as a common manifestation,[4,5] may correlate with consistent signal abnormalities in the basal ganglia and cerebellar dentate nuclei. The current natural history study in progress is designed to detect correlations between the imaging findings and clinical manifestations as well as metabolite concentrations from the accumulating biorepository.

Although GABA spectroscopy shows excellent discrimination of normal versus SSADH deficiency patients, edited MR spectroscopy is not a routinely available sequence outside of research centers at the present time, limiting its application in evaluating potential SSADH patients. However, routine proton MR spectroscopy does still have a role. Specifically, it can be used to exclude creatine deficiency syndromes (ie, GAMT deficiency) and signs of a mitochondrial disorder (ie, lactate).

The j-edited MEGAPRESS sequences allowed us to directly measure GABA; however, it should be noted that there is a small contribution of macromolecule signals, particularly those arising from spins at 3 ppm coupled to spins at 1.7 ppm that are affected by the editing pulses. This is an issue that all studies using MEGAPRESS must acknowledge as there are no well-documented solutions to the problem.[14]

Conclusion

Here in this work, we report our findings from an MRI study on a large cohort of SSADH deficiency patients, compared with age-matched controls. By using advanced spectroscopy techniques, we directly measured GABA in 3 different regions and show differences in GABA levels between SSADH deficiency patients and controls. We also report signal abnormalities in globus pallidus and cerebellar dentate nucleus, and also reduced cerebral volume.

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Declaration of Conflicting Interests

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Ethical Approval

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