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The Value of ^{1}H-MRS and MRI in Combined Methylmalonic Aciduria and Homocystinuria

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Objective: The aims of this study were to describe the brain magnetic resonance imaging (MRI) features of methylmalonic aciduria and homocystinuria and to evaluate the additional value of ^{1}H-MRS.

Patients and Methods: Twenty-eight children with methylmalonic aciduria and homocystinuria were included in this study. The control group included 21 healthy children. All the cases underwent MRI and ^{1}H-MRS before treatment. We measured the *N*-acetylaspartate (NAA), choline (Cho), creatine (Cr), and myoinositol (mI) peaks in the basal ganglia regions. The NAA/Cr, Cho/Cr, mI/Cr, and NAA/Cho ratios were calculated. We also observed whether there were lactic acid peaks.

Result: We identified that NAA/Cr and NAA/Cho significantly decreased in the basal ganglia and that 3 patients showed lactate peaks, but other metabolites were not significantly altered. Hydrocephalus and diffuse supratentorial white matter edema were the primary MR findings; 7 patients had thinning of the corpus callosum, and 2 patients had subdural hematoma. Six patients showed normal brain MRI findings.

Conclusions: Methylmalonic aciduria and homocystinuria patients with metabolite changes in the basal ganglia demonstrate compromised neuronal integrity, and anerobic metabolism occurs in acute encephalopathic episodes. ^{1}H-MRS is a useful tool for evaluating brain damage. Hydrocephalus and diffuse supratentorial white matter edema are the main MRI features.

Key Words: methylmalonic aciduria, homocystinuria, ^{1}H-MRS

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Combined methylmalonic acidemia and homocystinuria, which are inherited disorders of vitamin B12 metabolism, have been ascribed to mutations in MMACHC.[1] The defect causes deficiencies in adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), which are essential coenzymes for methylmalonyl-CoA mutase and methionine synthase, respectively. A decrease in these 2 enzymes' activity will lead to the accumulation of abnormal metabolites in body fluids. The early onset of this disease usually presents within 1 year of age and is accompanied by varying degrees of hematologic, gastrointestinal, and neurologic abnormalities. According to Huang et al[2] although Methylmalonic aciduria and Homocystinuria (MMA-HC) responds to combined treatment with folic acid, hydroxocobalamin, betaine, vitamin B6, and L-carnitine supplementation with biochemical and clinical improvement, the long-term outcomes are unsatisfactory, with neurological sequelae occurring in survivors. In contrast, patients with late onset show mild clinical symptoms dominated by acute neurologic dysfunction and psychomotor regression and have better outcomes.[3] Both biochemical and genetic approaches have been established to diagnose children and fetuses with MMA-HC.[4,5]

Plasma MMA and Hcy are routinely tested at clinical follow-up, but these biomarkers do not accurately reflect the neurodevelopmental state and may be poor predictors for outcomes. Magnetic resonance imaging (MRI) is a useful technique to evaluate brain development. So far, only a few neuroradiologic studies investigating MMA-HC have been reported.[6,7] However, sometimes it is difficult to identify chronic and acute injury at a cellular level based on traditional imaging. Proton magnetic resonance spectroscopy (^{1}H-MRS), which can be used to evaluate metabolism noninvasively at the cellular level, is one of the most advantageous methods to monitor and study neurometabolic disorders. Here, we present the MR findings in 28 patients with MMA-HC and discuss the clinical and brain MRI findings of the disease to assess brain damage via conventional MRI and to evaluate the additional value of ^{1}H-MRS.

PATIENTS AND METHODS

Patients

Between May 2013 and August 2015, 28 patients were diagnosed with MMA-HC by tandem mass spectrometry, gas chromatography–mass spectrometry, and genetic testing in our Department of Pediatric Endocrinology and Genetic Metabolism. The main clinical manifestations are lethargy, vomiting, developmental delay, and feeding difficulties. The participants included 15 males and 13 females, with an age range of 21 days to 7 years. The mean ± SD age was 15.03 ± 19.25 months. The patients' blood abnormalities were recorded as follows: C3 (propionylcarnitine): 9.64 ± 5.73 μmol/L, C3/C2 (propionylcarnitine/acetylcarnitine): 0.69 ± 0.32, MMA (methylmalonic aciduria): 305.31 ± 273.84 mmol/mmolCr, MGA (methylcitric acid): 13.6 ± 14.67 mmol/mmolCr, and Hcy (homocystine): 217 ± 128.34 μmol/L. All patients underwent MRI and ^{1}H-MRS before treatment. The control group consisted of 21 healthy children. There were 10 males and 11 females, with an age range of 30 days to 5 years, with a mean ± SD age of 18.78 ± 14.13 months. In all patients, the diagnosis was based on the findings of elevated homocystinuria, methylmalonic aciduria, and genetic testing results. The study was approved by the institutional review board of our hospital, and the requirement to obtain informed consent was waived.

Image Analysis

Magnetic resonance imaging scans were performed using a GE Signa 1.5T twin speed superconducting magnetic resonance

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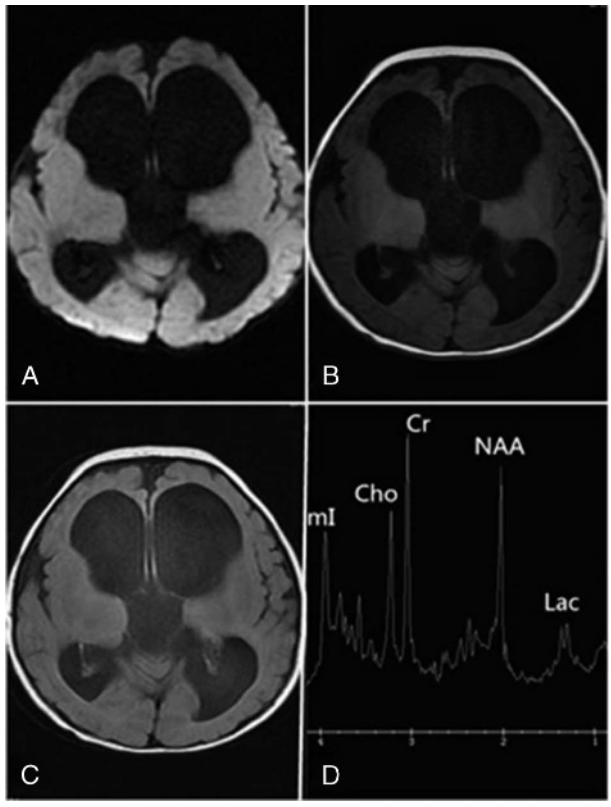

FIGURE 1. Case 1, M, 4 months. A, Axial DWI. B and C, Axial T1 and T2 fluid-attenuated inversion recovery, all sequence show obvious supratentorial ventricular dilatation and no abnormal signal in the bilateral basal ganglia. D, ^{1}H-MRS from the basal ganglia show Lac peak at 1.3 ppm.

instrument, and the patient's head was placed in a fixed sponge pad in an 8-channel head coil. Nineteen infants were sedated with 10% chloral hydrate at a dose of 0.5 mL/kg before the scan. The imaging protocol involved T1-weighted fluid-attenuated inversion recovery (T1-FLAIR; repetition time [TR], 2200 milliseconds; echo time [TE], 24 milliseconds; section thickness, 5 mm) and T2-FLAIR (TR, 8500 milliseconds; TE, 120 milliseconds; section thickness, 5 mm). Sagittal T2-weighted imaging (TR, 2200 milliseconds; TE, 90 milliseconds) and axial diffusion weighted imaging (DWI) (TR, 10,000 milliseconds; TE, 70 milliseconds, $b = 0$ s/mm^2 and $b = 1000$ s/mm^2)were also included in the scan. The MRI scans were analyzed by 2 experienced pediatric neuroradiologists with particular attention to the supratentorial white matter. The presence of hydrocephalus and the thickness of the corpus callosum were also evaluated.

^{1}H-MRS was acquired using the point resolved spectroscopy (PRESS) technique (TE, 35 milliseconds; TR, 1500 milliseconds) with 2-cm-sided voxels located in the right basal ganglia in each case. A procedure for the suppression of water signals and the interactive shim of the magnetic field across the volume of interest was used before spectral acquisition. The single voxel spectroscopy acquisition parameters were the following: field of view, 24–30 cm; TE/TR, 35/1500; scan time, 3 minutes 48 seconds. When the regions of interest were selected in the basal ganglia, the metabolite signals were analyzed as the ratios of the area under each peak: *N*-acetylaspartate (NAA)/creatine (Cr), NAA/choline (Cho), Cho/Cr, and myoinositol (mI)/(Cr). A comparison was made with a data set of ^{1}H-MRS metabolite concentrations, which was based on data from 21 healthy children.

Statistical analysis was performed using SPSS Statistics Version 19.0 (SPSS, Chicago, Ill). Continuous variables were analyzed using a nonparametric (Mann–Whitney) test. The correlation between the ratio of NAA/Cr and the blood parameters (Hcy, C3, C3/C2, MMA, and MGA) of the patient with MMA-HC were analyzed using Spearman correlation. All results are presented as the means ± SDs; $P < 0.05$ was considered significant.

RESULTS

Our patients showed different patterns of abnormalities, including hydrocephalus in 17 patients, supratentorial white matter edema in 8 patients, thinning of the corpus callosum in 7 patients, subdural hematoma in 2 patients, and completely normal findings in 6 patients. The main MRI features were supratentorial white matter edema and hydrocephalus (Fig. 1).

In 3 of 28 cases, ^{1}H-MRS showed the presence of a peak corresponding to Lac. Three patients had no lesions in the basal ganglia, but Lac was found in these regions (Figs. 1, 2). In the 25 remaining patients, Lac was not observed. In all the patients, the ratios of the integral values for Cho/Cr, NAA/Cr, NAA/Cho, and mI/Cr were compared with the normal ratios. As shown in Table 1, there were no correlations between NAA/Cr and any biochemical markers. NAA/Cr and NAA/Cho were significantly decreased in the basal ganglia, but no significant differences were found in Cho/Cr and mI/Cr between the groups (Table 2).

DISCUSSION

MMA-HC is thought to be the most common congenital abnormality in vitamin B12 metabolism. The clinical characteristics

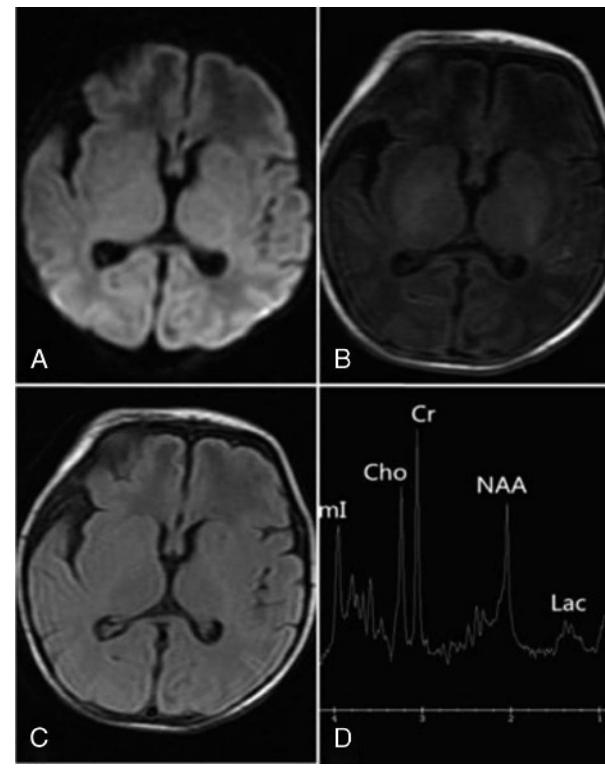

FIGURE 2. Case 2, M, 46 days. A, Axial diffusion-weighted imaging. B, Axial T1 fluid-attenuated inversion recovery sequence. C, Axial T2 fluid -attenuated inversion recovery sequence, all sequences showed bilateral lateral mild widened, and bilateral basal ganglia showed no abnormal signal. D, ^{1}H-MRS show Lac peak at 1.3 ppm.

TABLE 1. Analysis of Correlations Between NAA/Cr and Hcy, MMA, MGA, C3, and C3/C2

	NAA/Cr	
Biochemical Indicators	***r***	***P***
Hcy	0.351	0.057
C3	−0.173	0.361
C3/C2	−0.212	0.261
MMA	−0.273	0.145
MGA	−0.099	0.601

of the early-onset form, commonly occurring within 12 months of age, include varying degrees of neurological signs and gastrointestinal, hematological, and renal dysfunction.[8] However, the pathophysiology of MMA-HC is still unknown, but impaired methyl group metabolism, oxidative stress, and homocysteine accumulation may play significant roles.[9,10]

Consistent with the findings of previous reports,[6,7] we found that hydrocephalus and dilation of the lateral ventricle are the most common characteristics of the early-onset form. There is also diffuse supratentorial white matter swelling and thinning of the corpus callosum. The increased ventricular system is a nonspecific manifestation of neurological diseases affecting infant brain development. The classical hydrocephalus formation mechanism suggests that hydrocephalus occurs because of the imbalance in cerebrospinal fluid production and absorption. This absorption dysfunction may be due to cerebrospinal fluid flow disorders in the ventricular system, basal pool, cerebellar medulla, or spinal cord. This theory cannot completely explain the cause of hydrocephalus in children with MMA-HC. In this group of patients, the Hcy values were significantly increased. It has been reported in the literature that Hcy not only has damaging effects on vascular endothelial cells but also exhibits direct neurotoxic effects by over activating the *N*-methyl-D-aspartate receptor.[11] Therefore, we believe that the formation of hydrocephalus in children with MMA-HC is mainly caused by Hcy damage to intracranial arteries. This idea is consistent with a new theory of hydrocephalus formation proposed by Greitz et al[12] who believe that circulating cerebrospinal fluid is mainly absorbed through the surface of the spinal cord and the capillaries of the brain rather than by arachnoid granules and villi. The study further suggested that increased cerebrospinal fluid pressure can maintain blood vessels on the surface of the brain and spinal cord open, whereas the increase in cerebrospinal fluid pressure is due to arterial pulsations through the subarachnoid space.[12] Thus, any cause of reduced arterial compliance due to intracranial arteriosclerosis can cause hydrocephalus. The thinning of the corpus callosum may be caused by delayed or poor myelination. Studies have shown that poor myelination in MMA-HC is associated with a lack of S-adenosylmethionine in the brain as a result of metabolic abnormalities. Because S-adenosylmethionine is an important donor of methyl, its lack can lead to dysmyelination.[13] It is worth mentioning that white matter edema is also a common sign in this group of patients. In our patients, white matter edema often occurs in children who have not been treated in the early stage. The imaging findings are mainly marked by a high signal on T2-weighted imaging, and the gray matter is not clearly defined. On one hand, we believe that hyperammonemia caused by abnormal metabolism and the direct effects of toxic metabolites on brain cells are the main causes of white matter edema; on the other hand, white matter edema is also caused in part by the pressure of hydrocephalus on the surrounding white matter.

The basal ganglia, especially the globus pallidus, are highly metabolically active and are symmetrically influenced in metabolic abnormalities and toxic poisoning.[14] Interestingly in this study, none of our patients had any abnormal signals in the basal ganglia, which represents a special characteristic relative to propionic acidemia and isolated MMA. Propionic acidemia and isolated MMA are associated with an abnormal appearance in the basal ganglia due to cytotoxic edema and necrosis, which represents a distinctive feature.[15,16] However, previous studies also show basal ganglia lesions in MMA-HC. A patient with MMA-HC who developed progressive neurological deterioration with basal ganglia involvement was reported by Enns et al.[17]

Our data represent the largest series of children with MMA-HC to be investigated with ^{1}H-MRS. The basal ganglia can be affected in metabolic abnormalities, and the selective vulnerability of the basal ganglia in organic acidemia is well established. Our data provide further insight into the basal ganglia. ^{1}H-MRS is a noninvasive method to study metabolism in living creatures and can quantitatively detect brain metabolites at microscopic levels. Currently, ^{1}H-MRS has been widely used in studying central nervous system diseases.[18,19] Our results indicate that the NAA/Cr and NAA/Cho ratios in the basal ganglia of children with MMA-HC are lower than those in the normal control group. There was no significant difference in other metabolites between the 2 groups. We hypothesize that the reduction in NAA concentration in this study, in addition to suggesting brain neuron and axonal damage or functional changes, may occur because methylmalonic acid confers a developmental block on brain tissue aggregation. This hypothesis may require a larger sample to confirm. In children with MMA-HC in this study if there was obvious nerve and axonal injury in the brain due to MMA-HC, theoretically, the increase in the Cho peak and the decrease in the NAA peak should occur concurrently. However, the final statistical results showed no difference in the Cho concentration between the 2 groups. Therefore, this point again suggests that the reduction in the NAA peak in children is not only manifested as neuronal damage but also occurs because of the accumulation of abnormal metabolites, which interferes with the normal physiological metabolic processes in the brain and blocks the development of brain tissue. In addition, 3 patients with normal MR imaging of the basal ganglia also exhibited lactate peaks. This anerobic metabolism reflects basal ganglia ischemia or mitochondrial dysfunction. A lactate peak, which can also be seen in mitochondrial diseases, is not specific for MMA-HC.[20] However, the lactate peak is correlated with clinical performance, and patients who show lactate peaks are characterized by severe lethargy. In addition, we analyzed the correlation between the NAA/Cr ratio and the Hcy, MMA, MGA, C3, and C3/C2 ratios in children and found no significant

TABLE 2. Statistical Analysis of Sex, Age, and the NAA/Cr, Cho/Cr, mI/Cr, and NAA/Cho Ratios Between the Case Group and the Control Group

	Case Group	Control Group	*P*
Sex			
Male	15	10	0.776
Female	13	11	
Age	15.03 ± 19.25	18.78 ± 14.13	0.17
NAA/Cr	1.28 ± 0.26	1.59 ± 0.35	0.001
NAA/Cho	1.42 ± 0.35	1.68 ± 0.37	0.015
Cho/Cr	0.93 ± 0.26	0.96 ± 0.20	0.779
mI/Cr	0.34 ± 0.097	0.39 ± 0.09	0.059

correlations between the NAA/Cr ratio and any biochemical indicators. Therefore, we speculate that brain damage in children is not caused by the accumulation of a single abnormal metabolite but instead may be related to the duration of the disease.

Our study has some limitations. First, the time elapsed since the stage of the MMA-HC at which MRI examinations were performed varied among patients. Moreover, we only detected the metabolites on one side of the basal ganglia. In addition, our study population was small.

CONCLUSIONS

The clinical and imaging manifestations of patients with MMA-HC lack specificity. Relying on gas chromatography–mass spectrometry for the quantitative analysis of organic acids is key for diagnosis. However, brain MRIs can help clarify the pathological changes and the involvement of the brain. Functional sequencing and ^{1}H-MRS can provide more information related to brain tissue metabolism.

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