Cranial MR Spectroscopy of Tetrahydrobiopterin Deficiency

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BACKGROUND AND PURPOSE: **Severe and progressive neurologic disease remains a problem for patients with hyperphenylalaninemia due to a deficiency of tetrahydrobiopterin (BH4), even with early diagnosis and despite treatment with BH4 and neurotransmitter precursors. Few reports have included the associated imaging characteristics. Our purpose was to describe the imaging features of BH4-deficient patients identified by neonatal screening in a Taiwanese population and to correlate the imaging features with the treatment.**

METHODS: **This study analyzed the cases of eight BH4-deficient patients who were examined by MR imaging and MR spectroscopy. Analysis of the findings was correlated with the clinical findings.**

RESULTS: **One patient whose intelligence quotient score was lower than those of the other seven patients experienced seizures in conjunction with central white matter signal changes on MR images and a lactate peak on MR spectroscopy. Lactate peak was revealed in another patient who had marked elevations of *N*-acetylaspartate:creatine and *N*-acetylaspartate:choline ratios. Although most patients had a higher than average *N*-acetylaspartate:creatine or *N*-acetylaspartate:choline ratio, the patient who had decreases of both ratios possessed the highest intelligence quotient scores among the eight patients. In addition, the myoinositol:choline ratio correlated positively with the average BH4 dosage ($P = .027$, $r = 0.027$) and the choline:creatine ratio correlated negatively with the average 5-hydroxytryptophan dosage ($P = .035$, $r = -0.742$).**

CONCLUSION: **Compared with classical phenylketonuria, patients with BH4 deficiency have fewer white matter changes revealed by MR imaging but more changes revealed by MR spectroscopy. MR spectroscopy is a potential method with which to monitor the dosages of supplements used to treat this disorder. In addition, MR spectroscopy may be helpful in gaining understanding of the neurophysiological changes that occur in association with this disease.**

Mental retardation associated with hyperphenylalaninemia induced by mutations of the phenylalanine hydroxylase gene (EC 1.14.16.1) (defined as phenylketonuria) has been eradicated by early diagnosis and treatment of this disease (1). However, in those patients with tetrahydrobiopterin (BH4) deficiency, it is unclear whether severe and progressive neurologic disease can be prevented by early diagnosis and treatment with neurotransmitter precursors (levodopa and 5-hydroxytryptophan) (2–4). Neuroimaging studies, such as MR imaging, may have helped to evaluate brain damage in patients with BH4 deficiency, such as symmetrical calcifications in the lentiform nuclei, prominent sulci and fissures, subcortical cystlike lesions shown on T1-weighted images, and hyperintense lesions at the periventricular white matter shown on T2-weighted images (5– 9). Hyperintense white matter changes are also commonly seen in cases of phenylketonuria (10).

MR spectroscopy is a noninvasive method with which to measure brain metabolites. Previous MR spectroscopy studies of phenylketonuria revealed normal metabolite levels, except for increased phenylalanine levels (11, 12). Recently, low brain phenylalanine levels despite high stationary plasma phenylalanine levels were found to account for single cases of untreated patients of normal intelligence (13). Reports describing MR spectroscopic findings in cases of BH4 deficiency were scarce until now. Patients with BH4 deficiency usually have normal phenylalanine levels when receiving BH4 supplementation.

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MR imaging and spectroscopy data for individual patients affected by tetrahydrobiopterin deficiency

Patient No.	MR Images	Lactate Peak	NAA:CRE	NAA:CHO	NAA:MI	MI:CRE	MI:CHO	CHO:CRE	Patient Age (yr) at MR Imaging	Patient Age (yr) at IQ Test	MR Image Phe (μmol/L)	Average Phe (μmol/L)	VIQ	PIQ	FSIQ	Average Dosage (mg/kg/day) BH4	Average Dosage (mg/kg/day) Levodopa	5-HTP
1	White matter T2 ↑	+	1.259	1.442	1.444	0.872	0.999	0.873	8.5	7.7	177	119	64	55	56	1.72	8.30	1.43
2	Negative	–	1.553 ↑	1.389				1.118	8.5	6.9	108	103	84	86	83	1.20	4.04	0.65*
3	Negative	–	1.779 ↑	1.500	1.888	0.942	0.794	1.186	8.5	6.9	99	69	58	64	57	1.74	7.84	1.21*
4	Prominent sulcation	+	5.944 ↑	10.30 ↑	2.316	2.567	4.447	0.577	8	6.4	65	104	74	83	76	2.33	9.53	2.43
5	Prominent sulcation	–	1.487	2.659 ↑	0.799	1.860	3.328	0.559	5	5.3	45	104	78	82	78	2.92	10.53	2.19
6	Negative	–	0.645 ↓	0.681 ↓				1.523	5	4.0	83	102	92	106	98	2.25	9.35	1.49
7	Negative	–	1.692 ↑	3.192 ↑	4.577	0.370	0.697	0.530	5	3.6	6	106	80	95	92	1.94	9.02	2.64
8	Negative	–	1.452	1.525 ↑	1.248	1.163	1.222	0.952	4		74	62				2.31	9.42	2.07

Note.—NAA indicates *N*-acetylaspartate; CRE, creatine; CHO, choline; MI, myo-inositol; IQ, intelligence quotient; Phe, phenylalanine; VIQ, verbal intelligence quotient; PIQ, performance intelligence quotient; FSIQ, full-scale intelligence quotient; BH4, tetrahydrobiopterin; 5-HTP, 5-hydroxytryptophan.

* Patients 2 and 3 stopped 5-HTP supplementation at the ages of 7 and 6 years, respectively.

However, other changes in brain metabolites may be present, because many early treated cases still have low intelligence quotient scores (14).

During the past 10 years, by neonatal screening in a Taiwanese population, we identified 10 patients with hyperphenylalaninemia caused by the deficiency of 6-pyruvoyltetrahydropterin synthase (OMIM 261640), the most prevalent cause of BH4 deficiency (1). Our purpose was to describe the imaging features of those patients and to correlate the imaging features with the treatment. Another aim of this study was to investigate whether changes in brain metabolite concentrations in treated young BH4-deficient patients could be shown using proton localized MR spectroscopy.

Methods

Ten patients (including one sibling pair, patients 4 and 5) with BH4 deficiency were enrolled from January 1, 1991, to May 31, 2001, from a total of 1,337,490 screened neonates. Two patients who refused MR imaging were excluded. Diagnosis was made by urine pterine analysis and the BH4 loading test (2). Genotypic characterization at the 6-pyruvoyltetrahydropterin synthase locus was also obtained. These patients were initially treated by a standard protocol, including low phenylalanine diet, 3.3 mg/kg/day of BH4, 10 mg/kg/day of Madopar (200 mg of levodopa and 50 mg of benserazide), and 5 mg/kg/day of 5-HPT supplementation. The dosages were adjusted thereafter according to both body weights and side effects (1, 2). Plasma phenylalanine concentrations were determined by an amino acid analyzer monthly for the first 6 months of life, every 2 to 3 months for the next 18 months, and quarterly thereafter, and also on the day of MR imaging examination. Intellectual assessment was conducted using the Chinese version of the Wechsler Intelligence Scale for Children-Revised (15) after patients had reached the age of 3 years, except for patient 8 who refused assessment. A detailed description of the treatment outcomes for these patients is presented in another article (14).

All patients were examined with a 1.5-T imager (Magnetom Vision Plus; Siemens, Erlangen, Germany) and standard circularly polarized head coil for both imaging and spectroscopy. Fast spin-echo T2-weighted images were acquired first as the localizer for MR spectroscopy. Localized proton MR spectra were acquired using a short TE-stimulated echo acquisition mode technique (TE = 10 ms). The 8-mL volumes of interest were localized in the parieto-occipital periventricular white matter. The time domain signals were filtered by applying a 1-Hz exponential function for signal-to-noise improvement. Areas of the spectral peaks were calculated by software integrated with manual settings of the upper and lower limits. All peaks were sufficiently resolved, and no resolution enhancement filtering was necessary. To quantify possible changes in the proton nuclear MR spectra, the ratios of *N*-acetylaspartate (NAA):creatine (Cre), NAA:choline (Cho), NAA:myoinositol (MI), MI:Cre, MI:Cho, and Cho:Cre were calculated. Eight unrelated children between 5.0 and 8.5 years of age (mean age, 6.3 ± 1.8 years) who had undergone MR imaging for headache or frontal seizure disorder without obvious brain lesions served as control patients.

Risk factors for patients showing a low intelligence quotient score were sought from a list including the following: body weight at birth; first plasma phenylalanine test level; dose and timing of administered BH4, levodopa, and 5-hydroxytryptophan; plasma phenylalanine levels on day of MR imaging examination; and ratios of metabolites as revealed by MR spectroscopy. Statistical analysis was conducted using the Mann-Whitney U test and Pearson's product-moment correla-

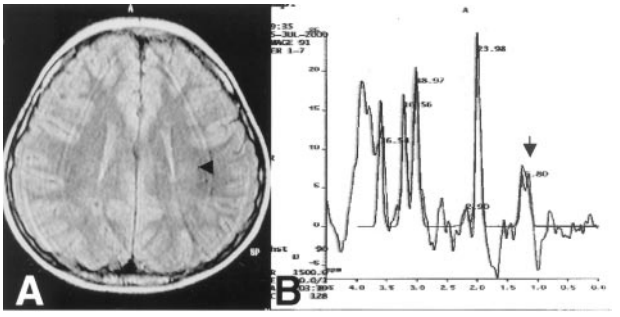

FIG 1. MR imaging findings and MR spectroscopic findings for patient 1.

A, Fluid-attenuated inversion recovery MR image (9000/110 [TR/TE]; inversion time, 2500 ms) of an 8-year-old male patient, obtained through the level of the centrum semiovale in the axial plane. Hyperintense areas (*arrowhead*) can be seen in the central white matter of the bilateral parietal cortex.

B, Proton MR spectroscopy sampled at the left parietal cortex and central white matter of the same patient. A duplex peak resulting from lactate can be seen at approximately 1.3 ppm (*arrow*), and active demyelination was suspected.

tion. All values are expressed as mean ± 1 SD (range). A value of $P < .05$ was considered significant.

Results

The findings of eight patients, 4.0 to 8.5 years old (mean age, 6.6 ± 1.9 years), who underwent MR imaging are summarized in the Table. These patients started a low phenylalanine diet at the age of 34 ± 13 days (range, 7– 50 days) and started BH4 and levodopa supplementation at the age of 1.9 ± 1.7 months (range, 0.2– 5.9 months). MR imaging disclosed abnormal high signal intensity in the cerebral white matter on T2-weighted images only for patient 1, who had the lowest full-scale intelligence quotient score and experienced seizures at age 6 months (Fig 1A).

MR spectroscopy disclosed lactate peaks in two cases: patients 1 (Fig 1B) and 4. Ratios of NAA:Cre, NAA:Cho, NAA:MI, MI:Cre, MI:Cho, and Cho:Cre were calculated and are summarized in the Table. Patient 4 had marked elevations of NAA:Cre and NAA:Cho. Most other patients (except patient 6) also exhibited elevated NAA:Cre or NAA:Cho ratios, although these were not statistically significant. On the contrary, patient 6, who possessed the highest verbal, performance, and full-scale intelligence quotient scores among the eight, revealed low NAA:Cre and NAA:Cho ratios. These patients also had higher MI:Cho ratios when compared with the control patients ($P = .020$). We failed to find any risk factors to predict poor intelligence quotient scores. However, the ratio of NAA:MI was positively correlated with birth body weight ($P = .048$, $r = 0.815$), the ratio of MI:Cho was positively correlated with the average BH4 dosage ($P = .027$, $r = 0.864$), and the ratio of Cho:Cre was negatively correlated with the average 5-hydroxytryptophan dosage ($P = .035$, $r = -0.742$). In addition, the ratio of MI:Cho was positively correlated with the ratio of MI:Cre ($P = .002$, $r = 0.966$).

Discussion

MR images have disclosed subcortical hypointense cystic lesions on T1-weighted images and hyperintense periventricular white matter on T2-weighted images of BH4-deficient patients, consistent with the spongiosis and dysmyelination shown by pathologic examinations (16). The increased signal intensity in the white matter regions on MR images may represent demyelination or dysmyelination. T2-weighted imaging white matter signal abnormalities are also common in phenylketonuria, and histopathologic studies have shown spongy degeneration of the myelin and foci of demyelination (17). Even in early treated phenylketonuria patients, cortical atrophy and regions of abnormal signal intensity could still be seen on MR images (9). However, in this study, high signal intensity in central white matter on T2-weighted images was observed in only one of the eight patients, which may indicate better treatment in our patients. The affected patient (patient 1) was initially treated only by dietary control, and levodopa and BH4 were administered after a seizure attack at 6 months of age. This might account for the patient's low intelligence quotient scores.

Lactate peaks occurred in patients 1 and 4. This finding was unexpected, because lactate is produced by anaerobic glycolysis but both patients were in stable condition when the MR imaging examinations were scheduled. Patient 1 tended to have higher protein intake and occasionally mild elevation of plasma phenylalanine levels, but the appearance of lactate peak has not been observed in classical phenylketonuria. However, in cases of BH4 deficiency, systemic administration of medications may not fit the requirement of neurotransmitters in different areas of brain. Therefore, the derangement in neurotransmitters may cause focal alteration in brain metabolism or active demyelination, in which lactate level elevates.

Many of our cases had elevated NAA:Cre and/or NAA:Cho ratios. In classical phenylketonuria, concentrations of NAA, Cre, and Cho are unchanged in the cerebral white matter in most studies (11, 12), although Dezortová et al (18) reported a group of 69 patients with classical hyperphenylalaninemia with increased NAA:Cho ratios and explained it by a decrease in Cho compounds. Both demyelination and cystlike lesions have been shown to occur in association with BH4 deficiency (16). If myelination is abnormal in development, the Cho levels may be lowered; during active demyelination, however, Cho levels may rise. The cystlike lesions, on the other hand, could lead to reduced Cre and then elevation of the NAA:Cre ratio. Although gross change in signal intensities in white matter was observed in only one case and no cystic lesion could be identified, mild subclinical changes were still possible in our patients. NAA is found only in neurons and axons, where it can be reduced and can thereby provide an index of irreversible damage, such as occurs in brain infarcts, brain tumors, epilepsy, multiple sclerosis, and neurodegenerative disease (19). Acute brain injury is one of

the few pathologic conditions associated with elevation of NAA. Therefore, it is possible that the elevation of NAA:Cre and NAA:Cho ratios in BH4-deficient patients may indicate continuous brain injury. The same process could induce the appearance of lactate peaks, and the prominent sulcation in two patients (patient 4 and 5) may represent the consequences of the injury. It is intriguing that the patient (patient 6) who had decreased NAA:Cre and NAA:Cho ratios seemed to be smarter than the other patients. She might have had a lesser degree of active brain injury, so the loss of neuronal tissues could have been reflected by the decrease of the NAA:Cre ratio.

For optimization of individual treatment, the dosage for BH4, levodopa, and 5-hydroxytryptophan should be closely monitored with objective measurements (13). In our study, a higher than average 5-hydroxytryptophan dosage correlated significantly with a lower Cho:Cre ratio, which may indicate less cell membrane destruction and could be an index for adequate 5-hydroxytryptophan therapy. Also, we observed that a higher than average BH4 dosage correlated significantly with a higher MI:Cho ratio. One possible explanation is that a higher than average BH4 dosage could stabilize cell membrane damage in the brain and therefore prevent or reduce Cho release. Another possible explanation is that MI concentration in these BH4-deficient patients is higher, which might be consistent with previously reported imaging findings of gliosis. Although the role of inositol in the metabolism of the brain is not clear, biosynthesis of inositol may increase when these BH4-deficient patients are supplied with a higher dosage of BH4, as was the case in our study. Our failure to correlate those ratios with intelligence quotient scores might have been due to the small number of cases investigated. More cases are therefore needed for further evaluation.

In conclusion, compared with classical phenylketonuria, patients with BH4 deficiency have fewer white matter changes revealed by MR imaging but more changes revealed by MR spectroscopy. MR spectroscopy is a potential method with which to monitor the dosage of supplements used to treat this disorder. In addition, MR spectroscopy may be helpful in gaining understanding of the neurophysiological changes that occur in association with this disease.

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