J Inherit Metab Dis (2006) 29: 127–134
DOI 10.1007/s10545-006-0080-y

ORIGINAL ARTICLE

Long-term outcome and neuroradiological findings of 31 patients with 6-pyruvoyltetrahydropterin synthase deficiency

L. Wang · W.-M. Yu · C. He · M. Chang · M. Shen · Z. Zhou · Z. Zhang · S. Shen · T.-T. Liu · K.-J. Hsiao

Received: 10 January 2005 / Accepted: 14 June 2005

Summary Tetrahydrobiopterin (BH_4) deficiency is an autosomal recessive disorder caused by enzyme defects in the biosynthesis or recycling of BH_4. Patients with BH_4 deficiency present with severe neurological signs and symptoms and require a different treatment from classical phenylketonuria. During the last 12 years, 31 cases of BH_4 deficiency were identified in our department. They were all classified as 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. They were diagnosed at the ages of 2.5–48 months and treated with BH_4, L-dopa and 5-hydroxytryptophan immediately after diagnosis. The average development quotients (DQ) at diagnosis and after treatment for more than 3 years were 53 ± 16, and 78 ± 15, respectively. A significant negative correlation was observed between the level of the DQ and the age at which treatment was commenced ($r = -0.751$, $p = 0.002$). Developmental profiles were uneven. Language, adaptability and at later age mathematics were particularly weak areas. Only two patients achieved a good performance in mathematics. Eleven patients who were treated with drugs from ages of 2.9–48 months had neuroradiological scanning. Computed tomography disclosed calcification in lentiform nuclei in one patient and magnetic resonance imaging disclosed delayed myelination and abnormal high intensity signal in cerebral white matter in all of them. Even though most of abnormalities were reversible, small patchy or spotted areas were still present on these regions after treatment for 10–46 months. In summary, our study supports the substantial efficacy of the current therapeutic approach in PTPS deficiency of normalizing amine neurotransmitters with three drugs as early as possible. For the first time, calcifications could be detected in patients with PTPS deficiency. Abnormalities in white matter on magnetic resonance imaging were not related to clinical manifestations and most were reversible.

Communicating editor: Georg Hoffmann

Competing interests: None declared

L. Wang · W.-M. Yu (✉) · C. He · M. Chang · M. Shen · Z. Zhou · Z. Zhang · S. Shen
Department of Medical Genetics, China–Japan Friendship Hospital, Yinghua East Road, Chaoyang District, Beijing 100029, China
e-mail: yuweim@public.bta.net.cn

T.-T. Liu · K.-J. Hsiao
National Yang-Ming University, Taiwan

Hyperphenylalaninemia (HPA) may be caused by mutations in phenylalanine-4-hydroxylase (PAH) or deficiency in tetrahydrobiopterin (BH_4), the essential cofactor required for the nitric oxide synthase (EC 1.14.13.39), phenylalanine (EC 1.14.16.1), tyrosine (EC 1.14.16.2) and tryptophan hydroxylases (EC 1.14.16.4). The latter two enzymes catalyse the rate-limiting steps in the biosynthesis of the monoamine neurotransmitter precursors, catecholamines and serotonin (Kaufman 1993). Most patients with BH_4 deficiency develop severe and progressive neurological impairments and require a different treatment from classical phenylketonuria (PKU; Mckusick 261600). Selective screening for BH_4 deficiencies is today an integral part of the newborn screening programme for HPA. So far, five defects—guanosine triphosphate-cyclohydroxylase (GTPCH; EC 3.5.4.16), 6-pyruvoyl-tetrahydropterin synthase (PTPS; EC 4.3.2.12), sepiapterin reductase (SR; EC 1.1.1.153), dihydropteridine reductase (DHPR; EC 1.6.99.7), and pterin-4a-carbinolamine dehydratase (PCD; EC 4.2.1.96)—are known to lead to dysfunction of BH_4-dependent hydroxylases, resulting in insufficient precursors for serotonin as well as catecholaminergic neurotransmitters. The most common form of BH_4-deficient HPA is PTPS deficiency (McKusick 261640) followed by DHPR deficiency

(McKusick 261630) (Blau et al 1996a; Liu et al 2001). Because BH_4 does not penetrate the blood–brain barrier well, supplementation with the amine neurotransmitter precursors levodopa and 5-hydroxytryptophan (5-HTP) is necessary to improve the turnover of dopamine and serotonin (Dhondt 1984). Although these drugs can effectively control symptoms such as disturbance of tone and seizures, it is not clear whether brain damage can be prevented completely. In 2001 Chien and colleagues had reported that their outcome was not as good as that for classical PKU, probably owing to inadequate treatment or prenatal brain damage. In this study, we describe the varying long-term outcome of 31 patients with PTPS deficiency and their neuroimaging findings to assess the efficacy of treatment and to obtain a better insight into the brain damage.

Materials and methods

Patients: From 1992 to 2004, a total of 559 patients, mainly from northern China, with HPA were investigated for BH_4 deficiency after informed consent from their parents in our clinics. Overall the data of patients with HPA and PTPS deficiency are as detailed in Table 1. Of these patients, only 159 cases were diagnosed as HPA by neonatal screening. The others were identified symptomatically when they came to our outpatient clinic with neurological symptoms such as hypotonia, mental retardation and seizures.

Differentiation of pterin defects from PAH deficiency: Urinary pterins were analysed by high-performance liquid chromatography (Niederwieser et al 1984; Blau et al 1992; Dhondt et al 1981), and DHPR activity in blood was measured as has been described previously (Curtius et al 1991).

Table 1 Summary data of patients with HPA and PTPS deficiency

Total number	559
HPA	
Neonatal screening	159
Symptomatic diagnosis	400
PAH deficiency	528
PTPS deficiency	31
PTPS deficiency	
Biochemical examination	31
Mutation analysis	8
Outcome of PTPS deficiency	
Under treatment	26
Refusal to treatment	2
Died	3
Follow-up of PTPS deficiency	
Anthropometry	26
DQ/IQ	26
Neuro-imaging	11

Further BH_4 loading test was performed by oral administration of BH_4 (6-R-BH_4, purchased from Schircks laboratories, Switzerland) at a dose of 20 mg/kg in patients with abnormal pterin profiles, and blood samples were obtained at time zero, and at 2 h, 4 h, 8 h and 24 h after BH_4 intake. Plasma phenylalanine concentrations were determined by HPLC (Blau 1996b; Niederwieser et al 1979; Ponzole et al 1993).

Management and follow-up: PTPS-deficient patients were treated with BH_4, L-dopa and 5-HTP together with carbidopa, an inhibitor of peripheral aromatic amino acid decarboxylase. The dosages usually given were BH_4 1–5 mg/kg (body weight) per day; L-dopa 5–15 mg/kg per day; and 5-HTP 3–10 mg/kg per day. Administration of the three drugs was started with small dosages and increased slowly. The optimal dosages were adjusted thereafter according to body weight and patients' reactions, especially the occurrence of side-effects. They were usually divided into three or four equal portions during the day to avoid the on–off phenomenon. Plasma phenylalanine concentrations were measured monthly for the first 6 months after treatment, every 2–3 months during the next 18 months, and quarterly thereafter. Clinical response was examined every 6 months. Intellectual assessments–development quotient (DQ) and later the intelligence quotient (IQ)—were carried out each year using the Gesell Child Developmental Age Scale and the Wechsler Intelligence Scale for Children–revised (WISC-R).

Neuroimaging: Computed tomography (CT) and magnetic resonance imaging (MRI) were performed in 11 patients whose parents could afford and agreed to take these examinations. Brain scans were done using 10×10 mm CT slice thickness/interval combinations and FOV $= 220$ mm (Picker 6000, Picker, Highland Heights, OH, USA). MRI scanning was performed with a 0.5 T imager (GE Signa) and standard circularly polarized head coil for imaging. Ten regions including frontal lobe, parietal lobe, occipital lobe, temporal lobe, cerebellum, corpus callosum, pons, midbrain, anterior and posterior limb of internal capsule were scanned. The myelination in ten sections was evaluated by using MRI T1-WI (TR $= 500$ ms, TE $= 15$ ms) and T2-WI (TR $= 4000$ ms, TE $= 80$ ms) with Staudt's standard by brain myelination in healthy children. Re-examination by MRI was performed on these patients after treatment for 10–46 months.

Statistical analysis: Statistical analysis was performed by comparing birth weight, birth length and head circumference of babies with PTPS and those of control newborns, by comparing phenylalanine levels in PTPS to those in PKU using the *t*-test, and by Pearson correlation analysis on long-term outcome with timing of treatment. All values are expressed as mean $\pm$ standard deviation (range). A value of $p < 0.05$ was considered significant.

Results

Patients with BH_4 deficiency: From 1992 to 2004, 559 patients with HPA were diagnosed and/or cared for in the China–Japan Friendship Hospital. Among these, a total of 31 cases were confirmed as suffering from BH_4 deficiency. These were 21 males and 10 females, including two pairs of siblings (cases nos. 389 and 390 and cases nos. 588 and 589). Consanguinity was not known in any of the families, 48 of the 58 parents were born in northern China, and more than 33% of them (20/58) originated from Jiaodong Peninsula, Shandong province.

Biochemical data, mutations, clinical presentation and outcome of treated patients are detailed in Table 2. Results of analysis of pterins all exhibited lowered biopterin and elevated neopterin. DHPR activity in red blood cells was always normal. After BH_4 loading, plasma phenylalanine levels dropped to normal within 4–8 h and tyrosine rose in 23 patients (Fig. 1a and b). Eight patients were confirmed by mutation analysis. In summary, PTPS deficiency could be ascertained in all of the 31 patients.

Among the 159 cases of HPA detected by neonatal screening, PTPS deficiency was the cause in 21 patients, giving a frequency of BH_4 deficiency as a cause of HPA of 13.2%. Compared to control children, these patients presented with a lower birth weight and reduced head circumference (3.1 ± 0.5 kg, 31.9 ± 2.2 cm, $p = 0.02$, 0.003 respectively; data not shown).

From the 31 patients diagnosed, specific therapy was started in 26 treated at ages 2.5–48 months. Twenty-eight of them presented with neurological signs linked to impaired catecholamines and serotonin synthesis when diagnosed symptomatically at the ages of 3.7–48 months. Most of them were considered normal during the first weeks of life, although signs and symptoms such as microcephaly, poor sucking, and decreased spontaneous movements may have been present. Abnormal development was recognized on average at age 4–5 months. The main symptoms were global developmental delay, disturbances of tonicity and posture, drowsiness, hypersalivation, swallowing difficulties and irritability. Ten patients exhibited abnormal movements at the age of 2.5–12.0 months such as opisthotonus or lead-pipe rigidity. Episodes of paroxysmal movements were considered of epileptic origin in 10 patients. Three of them had an abnormal electroencephalogram (EEG) that included spikes and sharp waves (Table 2). Only the patients nos. 616, 587 and 273 had no apparent clinical signs when they were diagnosed at ages 2.5, 2.7 and 9 months, respectively.

Plasma phenylalanine concentrations at diagnosis were 939 ± 481 μmol/L (range 181–2045); the concentration in the subgroup detected by neonatal screening was 748 ± 381 μmol/L (range 181–1370). The average phenylalanine concentration of PTPS-deficient patients was no different from that of patients with classical PKU (Table 3).

Treatment and follow-up: Three patients died of pneumonia before 1 year of age; another two refused to start treatment; the rest were treated with BH_4, L-dopa and 5-HTP. The treatment was commenced between the ages of 2.5 and 48 months. The final dosages were BH_4 1.1–3.3 mg/kg per day, L-dopa 5.8–12.5 mg/kg per day and 5-HTP 3.0–6.9 mg/kg per day. The duration of follow-up was 11–125 months. The blood phenylalanine concentrations were quickly lowered after BH_4 supplementation and all patients had normal phenylalanine concentrations without dietary treatment. Episodes of paroxysmal movements disappeared and the movement disorders were controlled by L-dopa. The DQ values at diagnosis and after treatment for more than 3 years were 53 ± 16 and 78 ± 15, respectively. A significant negative correlation was observed between the DQ and the age at which treatment was started ($r = -0.751$, $p = 0.002$) (Fig. 2). The patients who were diagnosed by neonatal screening had much higher DQ/IQ than those who were diagnosed symptomatically (88 vs 62, respectively). The former tended to catch up with their normal developmental milestones, while the latter exhibited mental retardation and abnormal behaviour, such as irritability, autism and aggression. All but the two latest-treated patients, nos. 544 and 636, became able to walk and talk: of 24 patients, 22 patients can walk normally, 19 can talk normally. However, developmental profiles were uneven in

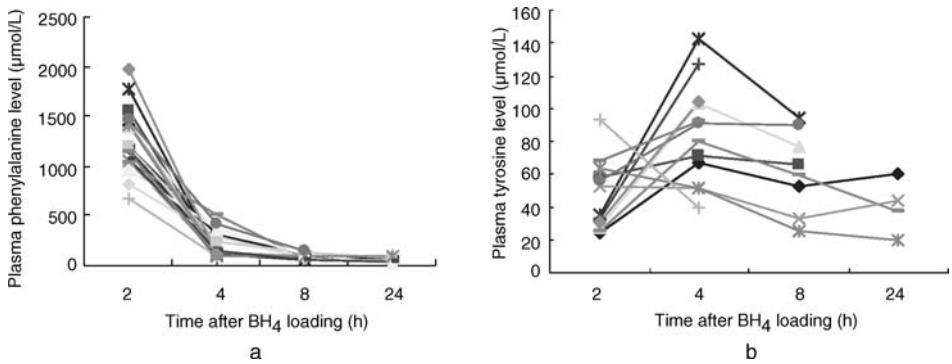

Fig. 1 BH_4 loading test of cases with abnormal pterin profiles. The plasma phenylalanine concentration decreased to normal while the plasma tyrosine rose slightly within 4–8 hours

Table 2 Biochemical and clinical findings and outcome of treated patients with PTPS deficiency

Patient no.	Age at diagnosis of HPA[a]	Initial Phe level (μmol/l)	Clinical findings			Age at diagnosis of PTPS (months)	Urinary pterins (nmol/L)		Confirmation test		Follow-up (months)	DQ/IQ		Medication (mm/kg per day)			MRI findings (delayed myelination)[c]
			Mental retardation	Tonicity	Movement disorders		N^b	B^b	Positive loading	Mutation analysis		At diagnosis	At report	BH_4	L-dopa	5-HTP	
163	10.3m, SD	1500	Severe	low	2.7m, abnormal EEG	10.3	6.10	0.21	+	/[c]	125	35	60	1.1	9.1	4.5	/
250	27.0m, SD	1080	Severe	low,	5m, abnormal EEG	27.1	n. r.	n. r	+	/	111	35	65	1.4	10.7	6.6	/
273	3d, NS	480	No	normal	/	9.0	4.31	0.12	/	166G>A/209T>A	104	97	112	3.0	7.5	3.0	/
389*	6d, NS	504	Severe	low	/	5.3	3.90	0.87	/	259C>T/286G>A	73	43	74	2.3	11.7	5.9	FL,OL,CC
390*	6d, NS	504	Severe	low	/	5.3	3.14	0.47	/		73	43	76	1.9	11.8	6.0	FL,OL,CC
405	7.2m, SD	1188	Severe	low	4m	7.3	31.3	0.42	+	/	69	33	57	2.5	12.5	5.4	FL,PL,OL
407	3d, NS	960	Moderate	low	/	4.5	2.26	0.04	+	/	67	57	93	1.6	11.5	6.9	/
419	3d, NS	181.2	Moderate	low	/	4.0	12.0	0.12	+	/	54	58	80	1.2	11.0	4.4	/
502	3d, NS	960	Severe	low	5m	10.5	3.05	0.18	+	/	49	44	70	2.1	10.5	6.3	/
516	3d, NS	240	Severe	low	3m	4.5	4.50	0.27	+	/	43	46	85	3.3	9.7	6.2	FL,TL,OL
529	4d, NS	1368	Severe	low	2m	5.0	5.40	0.05	+	/	41	48	83	1.5	12.0	6.0	FL,PL,TL,OL
536	3d, NS	780	Moderate	low	/	3.6	6.19	0.16	+	/	40	50	78	2.1	10.5	5.3	FL
544	47.4m, SD	1161	Severe	low	5m, abnormal EEG	47.5	19.9	0.01	+	/	38	23	61	1.6	7.5	3.8	FL, CL, CC
553	3.7m, NS	1042	Mild	normal	4m	3.8	3.75	0.46	+	/	36	68	89	1.5	10.0	3.3	FL, PL, OL, CC
569	3d, NS	600	Mild	normal	/	3.7	1.95	0.08	+	/	31	62	87	2.3	9.1	5.5	/
583	3d, NS	1200	Moderate	low	/	4.2	3.95	0.35	+	/	28	50	79	2.5	8.0	4.2	/
587	6d, NS	1200	No	normal	/	2.7	11.0	0.50	+	/	26	77	102	1.1	7.1	3.7	FL,OL
588*	7.2m, NS	960	Moderate	high	7m	7.3	5.32	0.14	+	/	25	63	80	1.8	8.5	4.3	FL,TL,OL,CC
589*	7.2m, NS	960	Moderate	high	/	7.3	7.33	0.43	+	/	25	63	80	1.8	8.4	4.2	FL,TL,CC
599	12m, SD	1564	Severe	low	/	13.4	4.22	0.04	+	/	24	35	65	1.2	5.8	3.2	/
608	3d, NS	678	Mild	normal	/	5.3	7.60	0.29	/	IVS1−291A>G/379C>T	19	67	115	2.0	8.1	4.1	/
616	6d, NS	1200	No	normal	/	2.5	1.50	0.08	+	155A>G/259C>T	16	96	110	1.5	7.5	4.0	/
636	4.4m, SD	2045	Severe	high	4.5m	4.5	4.08	0.08	/	317C>T/3G>A	15	25	65	1.7	8.3	4.1	/
642	3d, NS	780	Severe	low	2.5m	19.0	4.31	0.01	/	155A>G/379C>T	14	43	66	2.2	8.6	4.3	/
654	27d, NS	930	No	normal	/	2.1	11.8	0.03	+	/	12	85	83	2.0	7.5	3.8	/
683	3d, NS	1020	Moderate	low	/	6.2	4.90	0.12	/	259C>T/286G>A	11	55	74	2.0	7.8	3.9	/

[a]d = days, m = months; SD (months) = symptomatic diagnosis; NS (days) = newborn screening; [b]N = neopterin; B=biopterin; [c]CL, cerebellum; CC, corpus callosum; FL, frontal lobe; PL, parital lobe; OL, occipital lobe; TL, temporal lobe; [d]– indicates that this investigation was not performed; *Brothers; †Calcification; n.r. = no record (the result was lost)

Table 3 Initial plasma phenylalanine levels of patients with PTPS and classical PKU

	Plasma phenylalanine concentration (μmol/L)	
Disease	Mean ± SD (31 patients)	Mean ± SD (patients diagnosed by neonatal screening)
PTPS	938.8 ± 481.4 (n = 31)	747.7 ± 381.3 (n = 21)
PKU	978.6 ± 492.0 (n = 30)	808.8 ± 642.6 (n = 23)
p	$p > 0.5$	$p > 0.5$

almost every child (Table 4). Patients exhibited good memory and recitation compared to the relative inability to express themselves in language, relationships with normal children and, in later years, mathematics, and logical thinking. Only two patients, nos. 273 and 516, achieved good scores at mathematics. One was treated from the age of 4.5 months and was interested in a calculator by chance. He got more stimulation in mathematics from 1 year of age. He is now 4 years old and can count from 1 to 1000. Another patient started dietary and drug treatment at the ages of 3 months and 9 months old, respectively. She grew up within a good economic and educational environment. In order to avoid retardation, her family put her in to an environment of mathematics, literature and arts and instructed her to solve logical problems. After positive intervention of 9 years, she presented excellent performance in every aspect, such as playing the piano, writing and relationships, and recently won the gold medal of the Olympic Competition of Mathematics in Beijing.

Neuroimaging: Of 11 patients investigated, 9 of them started a low-phenylalanine diet at the age of 24–63 (46 ± 16) days and started BH_4, levodopa and 5-HTP supplementation at the age of 12.5–30.4 (21.3 ± 6.0) weeks (2.9 months to 7.0 months). The other two patients started drug supplementation at the ages of 8 months in one case and 48 months in the other.

Table 4 DQ in different aspects of 14 cases who had been treated for over 3 years

Aspects	DQ
Adaptability	76 ± 17
Gross motor	81 ± 12
Fine work	81 ± 16
Language	75 ± 16
Individual-social	79 ± 12

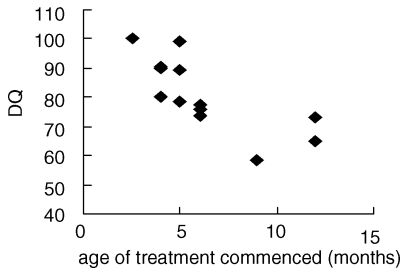

Fig. 2 Pearson correlation analysis of DQ and age of commencement of treatment of 14 cases who have treated for more than 3 years

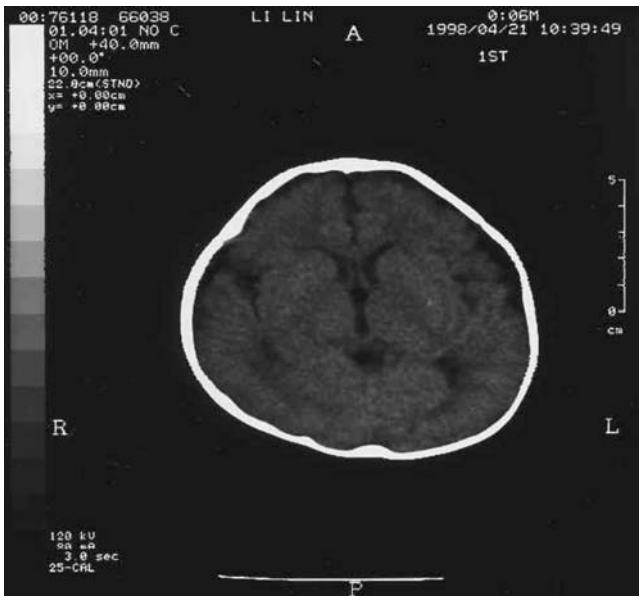

Fig. 3 Calcifications in the nucleus lentiformis on the right side of the brain. CT scan in patient no. 544 when he was 7 months old

Calcifications were observed on the right side of the brain CT in only one patient, no. 544, when he was 7 months old (Fig. 3). Their size was about 1.9 × 3.4 mm and they were located in the right nucleus lentiformis. On the initial MRI, widespread delayed myelination was seen in all patients, most pronounced in frontal lobe (11/11), followed by the occipital lobe (8/11), corpus callosum (6/11), parietal lobe (3/11), temporal lobe (4/11) and cerebellum (1/11) according to a modified staging system (Staudt et al 1994) (Table 2; Figs. 4 and 6). Abnormal high signal intensity in the periventricular area symmetrically extending to the occipital and the parietal lobes on T2-weighted imaging was present in all the patients (Fig. 4). The abnormal high signals in the white matter were all above grade 4 in the MRI grading system (Thompson et al 1991). After 10–46 months of treatment, major improvements were achieved, with disappearance of abnormalities in large areas of the frontal lobe, parietal lobe, occipital lobe and temporal lobe. Small patchy or spotted areas of abnormalities persisted in the frontal lobe (4/11), occipital lobe (4/11), corpus callosum (2/11), porietal lobe

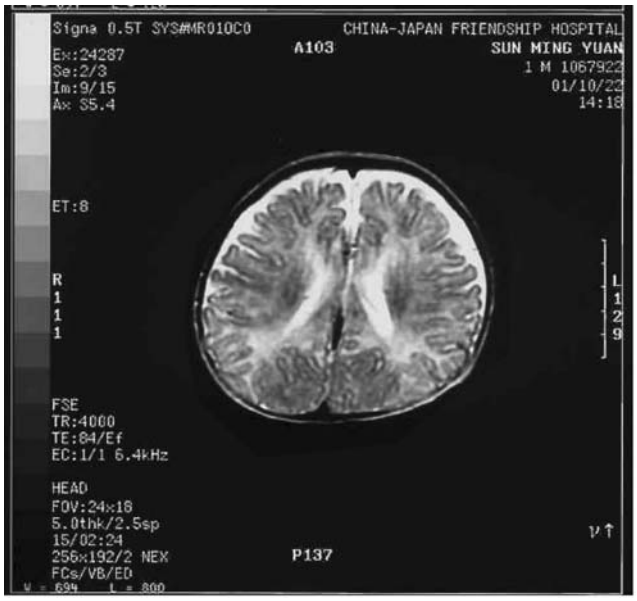

Fig. 4 Axial T2-weighted MRI scans through the brain of an infant (patient no. 553) at the age of 23 weeks. Abnormal high signal in periventricular areas

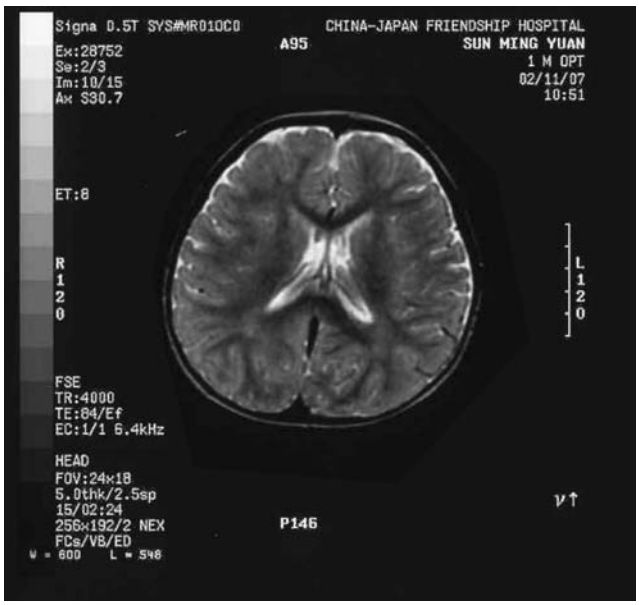

Fig. 5 After treatment for 11 months, most of the high signal abnormalities had disappeared. A single spot of high signal could still be detected

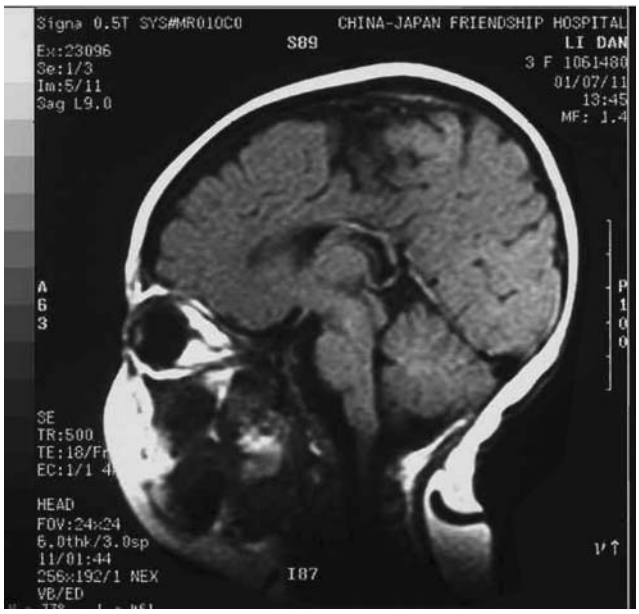

Fig. 6 Corpus callosum of a patient (no. 536, 3 months old) with hyperintensity in the splenium

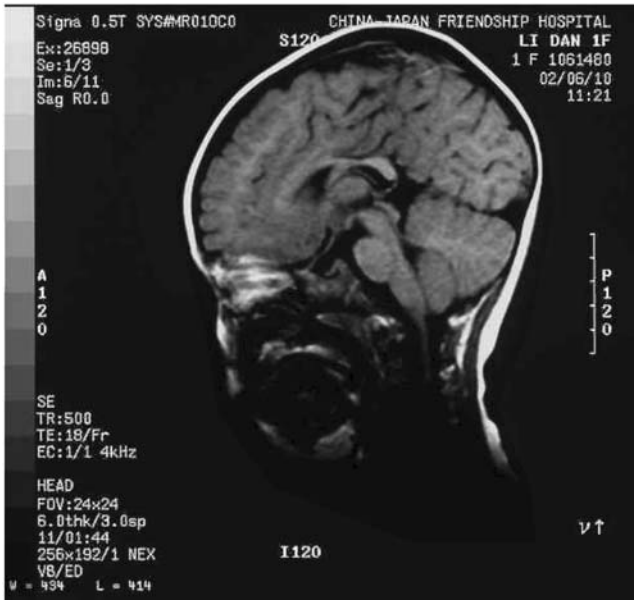

Fig. 7 After treatment for 13 months (at 16 months of age) the corpus callosum was not completely hyperintense

(1/11), temporal lobe (1/11) and cerebellum (1/11) (Figs. 5 and 7).

The abnormalities detected on MRI did not relate to the severity of neurological manifestations (Table 2).

Discussion

The Neonatal Screening Programme for PKU was introduced in the Peoples' Republic of China in the early 1980s and has had a rapid development in recent years. From 1985 to 2001, 5.8 million newborns have been screened; the incidence of PKU was 1 in 11 144 live births. The total coverage by the screening programmes was increased by 45.5% per year during the last 6 years and the cover age rate has reached more than 95% in large cities such as Beijing, Shanghai and Guangzhou.

Among the patients with hyperphenylalaninaemias presenting to our hospital during the last 12 years, 31 patients were diagnosed to suffer from a pterin defect leading to

BH_4 deficiency. The diagnostic work-up in our outpatient clinic revealed PTPS deficiency in all of them, which approximately accounts for 1/8 of such patients worldwide (www.bh4.org). It is an interesting observation that more than one-third of cases originated from Jiaodong Peninsula, Shandong Province in northern China. The regional high incidence of PTPS deficiency may be explained by founder effects such as have been described in Taiwan. Besides PTPS deficiency, the other pterin defects account for about 40% of BH_4 deficiency worldwide. However, no other cause such as DHPR or GTPCH deficiency has been identified in mainland China, until now. This is especially surprising for the area of southern China, which has a similar genetic background to Taiwan, where two patients with DHPR deficiency have been diagnosed.

Our data showed that phenylalanine concentrations at presentation vary over a large range from 181 to 1370 μmol/L. The average plasma phenylalanine concentration of patients with PTPS deficiency was not different from that of patients with classical PKU. Thus, plasma phenylalanine levels at presentation cannot help to differentiate PTPS deficiency from classical PKU. Pterin defects need to be differentiated in all infants with a positive neonatal screening result for phenylalanine. Most of the patients had already developed obvious neurological signs and symptoms when they were diagnosed in our clinic.

Factors that contribute to outcome: A lower than average birth weight and head circumference has been previously reported (Blau et al 1996a; Chien et al 2001). Although we treated all the patients with BH_4 and neurotransmitters combined immediately after diagnosis, the long-term outcome of our patients was not entirely satisfactory. The main negative factor was a delay in diagnosis in many patients. From analysis of correlation between the DQ/IQ and the age when treatment was started, we conclude that severe and progressive neurological disease can be prevented by early identification and treatment with BH_4 and neurotransmitter precursors. Moreover, the intelligence development was not uniform among different aspects. Active language and calculation appeared more compromised. The patients prefer to recite poems, stories on songs than to communicate with others and learn calculation skills. We carried out regular assessment of DQ/IQ to encourage the parents to maximize their potential.

Movement disorders: Some PTPS-deficient patients present opisthotonus or lead-pipe rigidity in the earlier stages of life. These symptoms were easily controlled by L-dopa. They were not seizures as in PKU patients, which cannot be controlled without administration of anticonvulsant drugs. These abnormalities should be extrapyramidal movement disorders with dystonia caused by disturbance of dopa.

Neuroimaging findings: Some reports on small numbers of patients with BH_4 deficiency (Brismar et al 1990; Chien et al 2002) have described neuroimaging findings such as symmetrical calcifications in the lentiform nuclei, subcortical cystlike lesions shown on T1-weighted images, and hyperintense lesions of the periventricular white matter shown on T2-weighted images, consistent with the spongiosis and dysmyelination revealed by pathological examination.

Of 11 patients investigated in our group, in one patient a calcification was detected in the nucleus lentiformis on the right side of the brain in CT scanning. Until now, calcifications have not been reported in PTPS deficiency but only in DHPR deficiency. This patient suffered from the most severe clinical manifestations among the 31 patients; he cannot stand up, walk or talk.

MRI revealed large areas of abnormalities in all patients investigated. These were not related to clinical findings. Nine patients had started a low-phenylalanine diet at the age of 46 ± 16 (24–63) days and commenced supplementation with BH_4, levodopa and 5-HTP at the age of 4.9 ± 1.4 (2.9–7.0) months. In 2002, Chien and colleagues reported MRI finding on eight patients with BH_4 deficiency who started a low-phenylalanine diet at the age of 34 ± 13 (7–50) days and started BH_4 and levodopa supplementation at the age of 1.9 ± 1.7 (0.2–5.9) months. Compared to classical PKU patients, these patients had fewer white-matter changes on MRI. Only one patient showed abnormal high signal intensity in the cerebral white matter. Our results are clearly different. While there was little difference in the ages at which dietary treatment was started (46 ± 16 vs 34 ± 13 days), the supplementation with BH_4 and neurotransmitter precursors occurred significantly later in our patient group (4.9 ± 1.4 vs 1.9 ± 1.7 months). It appears that such a delay contributed to the widespread delayed myelination. Most abnormalities were reversible after treatment for 10–46 months, with small patchy or spotted abnormalities remaining. Chien and colleagues also performed NMR spectroscopy and demonstrated elevated levels of several metabolites, such as *N*-acetylaspartate:creatine, *N*-acetylaspartate:choline, and lactate. A higher than average dosage of 5-HTP and BH_4 was recommended. The remaining focal abnormalities on MRI of our patients under treatment may be caused by inadequacies in the supplementation of neurotransmitter precursors and/or BH_4. It appears necessary to closely monitor metabolites in CSF and possibly the brain for optimization of BH_4, levodopa and 5-HTP individually.

In conclusion, this study supports the substantial efficacy of current therapeutic approaches in PTPS deficiency by supplementing with neurotransmitter precursors and BH_4 as early as possible and carefully monitoring development. Early therapeutic stimulation may help to maximize the developmental potential, including some weaker areas. Calcification could be detected in one patient with PTPS

deficiency. We propose that the finding of abnormalities in MRI merits further exploration of metabolites in the brain by MR spectroscopy for optimization of dosages of BH_4, levodopa and 5-HTP.

Acknowledgements The authors thank the patients and their parents as well as the physicians and nurses of the Department of Pediatrics for participation in the study. This study was supported by the National Natural Science foundation of China (NSFC no. 30271372).

References

Blau N, Barnes I, Dhondt JL (1996a) International database of tetrahydrobiopterin deficiencies. *J Inherit Metab Dis* **19**: 8–14.

Blau N, Kierat L, Heizmann CW, et al (1992) Screening for tetrahydrobiopterin deficiency in newborns using dried urine on filter paper. *J Inherit Metab Dis* **15**: 402–404.

Blau N (1996b) The hyperphenyalaninemias: a differential diagnosis and international database of tetrahydrobiopterin deficiencies. Marburg: Tectum Verlag.

Brismar J, Aqeel A, Gascon G, et al (1990) Malignant hyperphenylalaninemia: CT and MR of the brain. *AJNR Am J Neuroradiol* **11**(1): 135–138.

Chien Y-H, Chiang S-C, Huang A, et al (2001) Treatment and outcome of Taiwanese patients with 6-pyruvoyltetrahydropterin synthase (PTPS) gene mutations. *J Inherit Metab Dis* **24**: 815–823.

Chien Y-H, Pen S-F, Wang T-Rn, et al (2002) Cranial MR spectroscopy of tetrahydrobiopterin deficiency. *Am J Neuroradiol* **23**: 1055–1058.

Curtius HC, Blau N, Kuster T (1991) Pterins. In: Hommes FA, ed. *Techniques in Diagnostic Human Biochemical Genetics. A Laboratory Manual*. New York: Wiley-Liss, 377–396.

Dhondt JL (1984) Tetrahydrobiopterin deficiencies: preliminary analysis from an international survey. *J Pediatr* **104**: 501–508.

Dhondt JL, Largilliere C, Ardouin P, et al (1981) Diagnosis of variants of hyperphenylalaninemia by determination of pterins in urine. *Clin Chim Acta* **110**: 205–214.

Conoley JC, Impera JC (1995) Gesell Child Developmental Age Scale. The Twelfth Mental Measurements Yearbook.

Kaufman S (1993) New tetrahydrobiopterin-dependent systems. *Annu Rev Nutr* **13**: 261–286.

Liu TT, Chiang SHI, Wu SJ, et al (2001a) Tetrahydrobiopterin-deficienct hyperphylalaninemia in the Chinese. *Clin Chim Acta* **313**: 157–169.

Niederwieser A, Staudenmann W, Wetzel E (1984) High-performance liquid chromatography with column switching for the analysis of biogenic amine metabolites and pterins. *J Chromatogr* **290**: 237–246.

Niederwieser A, Curtius HC, Viscontini M, et al (1979) Phenylketonuria variants. *Lancet* 1979;1:550.

Ponzole A, Guardamagna O, Spada M, et al (1993) Differential diagnosis of hyperphenylalaninaemia by a combined phenylalanine-tetrahydrobiopterin loading test. *Eur J Pediatr* **152**: 655–661.

Thompson AJ, Smith T, Kendall BE, et al (1991) Magnetic resonance imaging changes in early treated patients with phenylketonuria. *Lancet* **377**: 1224–1228.

Staudt M, Schropp C, Staudt F, et al (1994) MRI assessment of myelination: an age standardization. *Pediatr Radiol* **24**: 122–127.

Wechsler D (1986) Wechsler Intelligence Scale for Children–Revised for China. Changsha, China: Human Medical University.