Long-term Follow-up of Taiwanese Chinese Patients Treated Early for 6-Pyruvoyl-Tetrahydropterin Synthase Deficiency

Kai-Ming Liu, MS; Tze-Tze Liu, PhD; Ni-Chung Lee, MD; Ling-Yee Cheng, MS; Kwang-Jen Hsiao, PhD; Dau-Ming Niu, MD, PhD

Objective: To report the long-term results of early initiation of treatment of 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency.

Design: Between 1988 and 2000, 12 newborns with PTPS deficiency who underwent early treatment at our hospital were identified. All patients received tetrahydrobiopterin replacement in a daily dosage between approximately 2 and 4 mg/kg. The dosages of levodopa replacement were 10 to 15 mg/kg/d, which is considerably higher than the typically recommended dosages of less than 7 mg/kg/d for patients aged younger than 2 years and 8 to 10 mg/kg/d for patients aged 2 years or older. Replacement with 5-hydroxytryptophan varied widely among patients.

Setting: Taipei Veterans General Hospital.

Patients: Twelve newborns.

Interventions: Treatment with tetrahydrobiopterin, levodopa, and 5-hydroxytryptophan.

Main Outcome Measure: IQ score.

Results: The mean (SD) IQ score of our PTPS-deficient patients was 96.7 (9.7; range 86-119), which is considerably higher than previous reports of other populations of PTPS-deficient patients. All patients reached a normal IQ on high daily dosages of levodopa replacement, without developing apparent long-term levodopa-induced adverse effects. We also observed a correlation between long-term IQ score and genotype, birth weight, and age at initiation of treatment.

Conclusions: An effective newborn screening referral program and early initiation of appropriate therapy preserved the IQ scores of PTPS-deficient patients.

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HYPERPHENYLALANINEMIA is the most common inherited disorder of amino acid metabolism. It may be caused by a deficiency of phenylalanine hydroxylase or tetrahydrobiopterin, an important cofactor involved in the biogenic syntheses of tyrosine, levodopa, 5-hydroxytryptophan, nitric oxide, and glycerol (Figure). Tetrahydrobiopterin deficiency may be caused by defects in the enzymes involved in its biosynthesis or in its regeneration. In white individuals, the overall prevalence of hyperphenylalaninemia attributable to tetrahydrobiopterin deficiency is only 1% to 2% of all cases. According to the International Database of Tetrahydrobiopterin Deficiencies database, which includes patients of various races, 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency (OMIM 261640) represents approximately 60% of all tetrahydrobiopterin deficiencies. 6-Pyruvoyl-tetrahydropterin synthase deficiency in humans may not only produce the typical phenylketonuric phenotype but may also be the source of neurological signs and symptoms due to impaired syntheses of levodopa and serotonin. 6-Pyruvoyl-tetrahydropterin synthase deficiency’s extrapyramidal manifestations, including, among others, truncal hypotonia, increased limb tone, postural instability, hypokinesia, choreic or dystonic limb movements, gait abnormalities, hypersalivation, and dysphagia, may resemble the signs of Parkinson disease. The disease is treated by tetrahydrobiopterin, levodopa, and 5-hydroxytryptophan replacement. However, choosing the proper amounts of precursors of neurotransmitters for replacement is challenging. While lumbar puncture is key in the diagnosis and monitoring of pediatric neurotransmitter disease, the choice of dosages of these precursors based on the concentrations.
of neurotransmitter metabolites in the cerebrospinal fluid might not always be optimal, as the patient’s metabolism might not systematically reflect the clinical status of PTPS-deficient patients. In addition, the invasiveness of lumbar puncture limits its serial use in routine clinical practice. There are few outcome studies of patients undergoing early treatment for PTPS deficiency, particularly over long periods of observation. Several reports have described adverse outcomes in a large percentage of patients with PTPS deficiency, despite its detection by newborn screening and the institution of early treatment.13,14

In Taiwan, where the disease’s prevalence (1 in 132,000) is considerably higher than in white individuals (1 in 1,000,000), PTPS deficiency is the cause of approximately one-third of all cases of hyperphenylalaninemia. A recent study at another Taiwanese medical center reported a mean (SD) IQ score of 76 (14) in 10 patients with PTPS deficiency detected by newborn screening.14 In contrast, we found a significantly higher (P < .001) mean (SD) IQ score (96.7 [9.7]) in 12 patients whose siblings were known to be PTPS deficient. A prenatal diagnosis was made in 2 other fetuses whose siblings were known to be PTPS deficient. The diagnosis of all patients identified by newborn screening was confirmed by (1) a tetrahydrobiopterin loading test, (2) analysis of urinary pterins, (3) enzyme assay of dihydropteridine reductase, and (4) mutational analysis of the PTS gene.

**TREATMENT**

Treatment with (1) tetrahydrobiopterin, (2) levodopa with a decarboxylase inhibitor, and (3) 5-hydroxytryptophan was initiated after confirmation of the diagnosis of PTPS deficiency. The administration of each neurotransmitter was based on the clinical response and the development of adverse effects observed during ambulatory follow-up. The initial dosage of tetrahydrobiopterin was approximately 3 to 4 mg/kg/d and was subsequently adjusted to keep serum phenylalanine concentrations below 120 µM. The initial dosage of levodopa with a decarboxylase inhibitor was 2 mg/kg/d, then increased every 2 to 5 days in 1-mg increments to a target dosage of 10 to 15 mg/kg/d. Beginning in 1996, serum prolactin concentration was measured at 1-month intervals in patients younger than 6 months and at 3-month intervals in older patients to guide the dosage of levodopa. When the concentration of serum prolactin exceeded 888 µg/L, the levodopa dosage was gradually increased until prolactin returned to less than 888 µg/L. However, in the absence of clinical manifestations of levodopa insufficiency, a dosage greater than 15 mg/kg/d was never administered, even in the presence of a persistently elevated prolactin. In the event of irritability or dyskinesia, the dosage of levodopa was lowered for several days, then increased again more slowly to the target dosage. 5-Hydroxytryptophan was initially administered in a dosage of 1 mg/kg/d, then increased every 2 to 5 days in 1-mg increments to a 5 mg/kg/d target dosage. As with levodopa, the dosage of 5-hydroxytryptophan was lowered when nausea, vomiting, diarrhea, or abdominal pain developed, then was slowly increased to the target maintenance dosage. Levodopa and 5-hydroxytryptophan were administered together in 4 divided doses before meals.

**METHODS**

Between 1988 and 2001, 10 screened newborns found to have elevated serum phenylalanine concentrations were referred to Taipei Veterans General Hospital and confirmed to have PTPS deficiency. A prenatal diagnosis was made in 2 other fetuses whose siblings were known to be PTPS deficient. The diagnosis of all patients identified by newborn screening was confirmed by (1) a tetrahydrobiopterin loading test, (2) analysis of urinary pterins, (3) enzyme assay of dihydropteridine reductase, and (4) mutational analysis of the PTS gene.
Table 1. Demographic and Biochemical Characteristics and Outcomes of Treated Patients With PTPS Deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Initial Phe Peak, µmol</th>
<th>BW, g</th>
<th>Gestational Age, wk</th>
<th>BW Percentile</th>
<th>Age at Treatment Onset, d</th>
<th>VIQ</th>
<th>PIQ</th>
<th>FIQ</th>
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<tr>
<td>1</td>
<td>P87S/N52S</td>
<td>1381.9</td>
<td>2750</td>
<td>38</td>
<td>32.92</td>
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<td>19</td>
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<tr>
<td>2</td>
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<td>2775</td>
<td>38</td>
<td>35.84</td>
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<td>148</td>
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<tr>
<td>3</td>
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<td>2700</td>
<td>37</td>
<td>41.38</td>
<td>27</td>
<td>90</td>
<td>27</td>
<td>11.3</td>
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<td>4</td>
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<td>40</td>
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<td>3300</td>
<td>41</td>
<td>56.5</td>
<td>34</td>
<td>55</td>
<td>48</td>
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<td>2780b</td>
<td>38</td>
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<tr>
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<td>33.16</td>
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<td>Mean</td>
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<td>2669</td>
<td>38.6</td>
<td>29.0</td>
<td>18.9</td>
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<td>SD</td>
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<td>349</td>
<td>1.6</td>
<td>17.1</td>
<td>8.8</td>
<td>104.1</td>
<td>11.7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Abbreviations: BH4, tetrahydrobiopterin; BW, birth weight; FIQ, full-scale IQ; Phe, phenylalanine; PIQ, performance IQ; PTPS, 6-pyruvoyl-tetrahydropterin synthase; VIQ, verbal IQ; 5-HTP, 5-hydroxytryptophan.

a Patients 2 and 10 were excluded because they were detected by prenatal diagnosis.
b Patients 6 and 7 were excluded because they are dizygotic twins from different families.
c Patient 10 was excluded because his mother gave his BW from memory, not proved by document. However, if we included this patient, we would get a more significant statistical correlation (Pearson $r = 0.651$; $P = .02$).

MEASUREMENTS OF INTELLECTUAL FUNCTION

IQ was measured by the Leiter International Performance Scale or the Wechsler Intelligence Scale for Children–III in children between the ages of 3 and 6 years of age, the Wechsler Adult Intelligence Scale, Revised, or the Wechsler Adult Intelligence Scale–III in patients older than 16 years.

Correlations between IQ scores and several variables, including genotype, peak serum concentration of phenylalanine and urinary concentrations of pterin at the time of diagnosis, birth weight percentile, and age at initiation of treatment with tetrahydrobiopterin, levodopa, and 5-hydroxytryptophan, were also analyzed in this study.

STATISTICAL ANALYSES

All values are expressed as mean (SD). Correlations between IQ and several variables were examined using the Mann-Whitney test and Pearson correlation. $P < .05$ was considered statistically significant.

RESULTS

Demographic characteristics, results of biochemical tests and mutation analyses, and IQ outcomes of our study population are detailed in Table 1. The mean duration of follow-up was 11.7 (3.3, range, 7.2-19.0) years. The mean final full-scale IQ of the 12 patients was 96.7 (9.7). Patients 2 and 10 had PTPS deficiency diagnosed before birth, and patients 7 and 10 are siblings. Excluding patients 2 and 10, the mean initial peak serum concentration of phenylalanine was 2249 (709) µM, which is considerably higher than in Taiwanese patients in whom hyperphenylalaninemia is caused by phenylalanine hydroxylase deficiency (798 [534] µM; $P < .05$).14 The mean age at diagnosis was 20.0 (6.3) days. Patients 6 and 7 are dizygotic twins from different families. Because twins' body weights are regularly low at birth, we excluded these 2 patients from the birth weight analysis. The mean birth weight was 2669 (349) g, which is significantly lower than in Taiwanese patients in whom hyperphenylalaninemia is caused by phenylalanine hydroxylase deficiency (3447 [492] g; $P < .001$).14 The allele frequencies were 54.2% (13 of 24) for N52S, 35.7% (9 of 24) for P87S, 4.2% (1 of 24) for R25G, and 4.2% (1 of 24) for F40L.

TREATMENT

All patients maintained serum concentrations of phenylalanine below 120 µM with a 2 mg/kg/d replacement dosage of tetrahydrobiopterin. Replacement with 10 to 15 mg/kg/d of levodopa was well tolerated by all patients. Treatment with levodopa caused occasional irritability and dyskinesia during the early part of replacement in the neonatal period, though they rarely occurred thereafter. In contrast, general hypotonia with ptosis, drooling, slurred speech, drowsiness, or nausea occurred regularly when replacement with levodopa was delayed or omitted. Nausea, vomiting, diarrhea, and abdominal pain occurred regularly during replacement with 5-hydroxytryptophan. In 1993, a shortage of 5-hydroxytryptophan in Taiwan forced the interruption of treatment for up to 6 months in our patients. Because no significant neurological change was noted during that interruption, or because of fear of adverse effects of 5-hydroxytryptophan, some of our patients abandoned this replacement or lowered their dosages to less than 2 mg/kg/d without apparent adverse neurological or intellectual consequences. Most patients were prescribed levodopa and 5-hydroxytryptophan to be taken in 4 divided doses per...
However, some patients changed their schedule to doses twice daily to avoid having to take medication while at school, without experiencing an on/off phenomenon or adverse neurological manifestations. This is in contrast with our observations in PTPS-deficient patients whose treatment was delayed in whom the on/off phenomenon occurred more commonly when they changed their medication schedule to 2 doses per day. The dosages of levodopa and 5-hydroxytryptophan administered to our patients at the ages of 2 months, 1 year, and the age at last follow-up are summarized in Table 2.

### FACTORS RELATED TO IQ OUTCOME

#### Genotype-Phenotype Correlation

The 3 mutations identified in this study (N52S, P87S, and R25G) have been observed to cause prominent phenotypical expressions of PTPS deficiency. However, their various combinations were associated with variably prominent expressions of the phenotypes. For example, before treatment, the patients with N52S/N52S or P87S/R25G genotypes whose treatments were delayed had the most prominent phenotypical disease manifestations. These patients were all bedridden and in a vegetative state before treatment initiation. On the other hand, in patients with N52S/P87S genotypes whose treatments were delayed, the disease manifestations before treatment onset were milder. They could eat, walk (albeit with an unstable gait), and articulate several single words, such as papa or mama. We also found that the patients with N52S/P87S genotypes who underwent early treatment had higher mean IQ scores (101.4 [9.8]) than patients who had other genotypes (90.0 [4.7]; P = .03).

#### Correlation Between Biochemical Measurements and IQ

After the exclusion of patients 2 and 10, we found no correlation between the initial peak phenylalanine concentrations and the full-scale IQs of our patients (Pearson r = −0.421; P = .23). Because PTPS deficiency impairs the synthesis of tetrahydrobiopterin and its metabolites, we examined the correlation between initial urinary bipterin concentrations and full-scale IQ, though we found none (Pearson r = 0.045, P = .9).

#### Relationship Between Birth Weight Percentile and IQ

After adjustment of the birth weight to the gestational age to calculate the birth weight percentile compared with the standard birth weight curves of healthy babies or twins (for patients 6 and 7) in Taiwan, we found a correlation between the percent birth percentile and full-scale IQ (Pearson r = 0.642; P = .03).

#### Relationship Between Treatment and IQ

No correlation was found between age at the time of onset of treatment with tetrahydrobiopterin, levodopa, or 5-hydroxytryptophan and full-scale IQ (tetrahydrobiopterin, Pearson r = 0.170, P = .6; 5-hydroxytryptophan, Pearson r = −0.021, P = .95; levodopa, Pearson r = 0.069, P = .83). Finally, we found no correlation between dosages of levodopa or 5-hydroxytryptophan administered at ages 2 months, 1 year, or at the last follow-up and full-scale IQ (Table 2).

### COMMENT

Our study indicates that (1) an effective referral system based on neonatal screening and (2) early appropriate therapy can preserve a normal IQ in PTPS-deficient patients. Therefore, similar disorders of neurotransmitters, such as aromatic L-amino acid decarboxylase deficiency, that are systematically associated with a poor prognosis might have better outcomes if they were treated early.
Unexpectedly, we found no correlation between age at the time of onset of treatment with tetrahydrobiopterin, levodopa, or 5-hydroxytryptophan and full-scale IQ. However, because all our patients were very young when treatment was initiated, the range of ages at the time of treatment initiation was narrow. Therefore, we repeated the analysis after the addition of data from 10 patients from another Taiwanese hospital’s report (age range at onset, tetrahydrobiopterin, 0.2-7.4 months; levodopa, 0.2-5.8 months; and 5-hydroxytryptophan, 1-57 months). This new analysis revealed correlations between age at onset of each medication and full-scale IQ (tetrahydrobiopterin, Pearson r = -0.655, P = .001; 5-hydroxytryptophan, Pearson r = -0.780, P < .001; levodopa, Pearson r = -0.645, P = .002). This observation suggests that the IQ did not vary significantly among PTPS-deficient patients whose treatment was initiated within 1 month of age, whereas persistent encephalopathy was likely to have developed in patients whose treatment was initiated later.

While there is little doubt that the early initiation of treatment can improve long-term outcome, there is no consensus with respect to the dosages of neurotransmitters to be administered to PTPS-deficient patients. An on/off phenomenon and levodopa-induced dyskinesia often develop during long-term therapy with levodopa in patients with Parkinson disease, and similar fluctuations in motor function and dyskinesia have also been observed frequently in PTPS-deficient patients. Some caregivers have abandoned the administration of high dosages of levodopa in favor of low daily replacement dosages because of frequent dyskinesia. In our experience, an insufficient daily replacement with levodopa facilitates the development of the on/off phenomenon. Unlike in patients with Parkinson disease, in our patients, dyskinesia regularly disappeared after a period of accommodation. This indicates that, in PTPS-deficient patients, dyskinesia is more likely caused by hypersensitivity of the end organs than by the peak-dose or biphasic effect observed in Parkinson disease. In Parkinson disease, the synthesis, storage, and reuptake of levodopa in the nigrostriatal system is impaired owing to the loss of dopaminergic neurons in contrast with PTPS-deficient patients, in whom only the synthesis of levodopa is impaired. This may explain the lower incidence of fluctuations in motor function and levodopa-induced dyskinesia in our early-treated patients than in patients with Parkinson disease undergoing prolonged therapy. Therefore, we believe that every effort should be made to continue neurotransmitter replacement therapy, because it is a reliable means of gradually overcoming the end-organ hypersensitivity.

While we found no correlation between the dosages of neurotransmitter replacement at different ages and full-scale IQ, it is noteworthy that, except for the dosage of patient 1 at the age of 2 months, the dosages of levodopa administered to our patients were consistently higher than the recommended dosages (<7 mg/kg/d for patients younger than 2 years of age and 8-10 mg/kg/d for older patients). This absence of correlation might thus be limited to the high range of dosages. On the other hand, some of our patients received 5-hydroxytryptophan late or in very low dosages, and some discontinued it for several years. Therefore, the absence of correlation between 5-hydroxytryptophan dosage and full-scale IQ might indicate that replacement with this drug was not an important determinant of full-scale IQ in our PTPS-deficient patients. Consequently, we believe that replacement with 5-hydroxytryptophan in the recommended daily dosages is not indispensable, particularly when major adverse effects develop.

Given that our patients are all Taiwanese Chinese, some patients from other populations might have even more severe genotypes. Consequently, their response to treatment might be different than that observed in our patients. Furthermore, because our study did not include a control group treated with low daily dosages of levodopa, we cannot prove that replacement with high dosages of levodopa improved our patients’ clinical outcomes. However, all our early-treated patients had favorable outcomes, and we observed no adverse effects caused by the long-term administration of levodopa in high daily dosages. We hope that these observations will prompt other caregivers to reconsider the dosages of neurotransmitters, with the goal of optimizing the clinical outcomes of PTPS-deficient patients.

The systematically lower mean birth weight of patients presenting with PTPS deficiency than that of normal individuals suggests prenatal effects of PTPS deficiency. The positive correlation between birth weight and IQ observed in our study also raises the issue of a possible prenatal effect on the brain development of PTPS. However, because of the many factors confounding IQ, the correlation between birth weight and IQ in our patients remains uncertain, particularly in light of (1) the small number of observations and (2) the presence of an outlier in this analysis (patient 4) whose birth weight percentile was 0.36. We believe that more data, contributed by multiple medical centers, are needed before a conclusion can be drawn regarding a possible correlation between birth weight and IQ in PTPS-deficient patients.

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REFERENCES


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