

## Original Articles

# Pitfalls in the Management of Phenylketonuria in China

LL YANG, HQ MAO, WF ZHANG, ZY ZHAO, RL YANG, XL ZHOU, XL HUANG, XW HUANG

### Abstract

To review the cognitive outcome of phenylketonuria (PKU) patients who were diagnosed through the newborn screening program. The management and treatment procedures were also evaluated. All PKU files during the period from January 1999 to September 2010 were reviewed and evaluated. The demographic information of the patients, results of average phenylalanine (Phe) concentration in the first three years, Phe concentration at the most recent clinic visit and neuropsychological assessment were recorded in detail. A total of 3,791,538 newborns had been screened for PKU in our centre. PKU was diagnosed in 147 children after newborn screening with an incidence of 1/25,600. After diagnosis, 24 children were refused to be treated by their parents. Four children were discontinued treatment after a period by their parents and nine were lost during the follow-up. Only 96 children have been followed up and treated at our centre until now. Neuropsychological assessments were only done in 55 patients, 11 of whom had mental retardation. The outcome of the children with delayed diagnosis and treatment was poor. Non-compliance to the treatment was common in PKU patients. Urgent measures have to be done to improve the screening strategies. Future study should focus on investigating the possible factors resulting in poor or suboptimal compliance, so as to improve the outcome of the PKU children.

### Key words

Management; Mental retardation; Phenylketonuria

### Introduction

Phenylketonuria (PKU; OMIM 262600), is an inborn error of phenylalanine (Phe) metabolism caused by a

deficiency of phenylalanine hydroxylase (PAH; EC 1.14.16.1), which synthesizes tyrosine from Phe, using tetrahydrobiopterin (BH<sub>4</sub>) as a cofactor. PAH deficiency causes Phe accumulation in the blood and other tissues of the affected patients.<sup>1</sup> The persistent high blood phenylalanine exposure particularly in untreated PKU patients will lead to mental retardation or delayed cognitive development, growth abnormalities, and other neuropsychological complications.<sup>2-4</sup> Early detection by newborn screening and timely treatment may result in near normalisation of outcomes of individuals with PKU. The current therapy of PKU consists of Phe intake restriction, a diet with low natural protein content supplemented with a special Phe-free formula, enriched with tyrosine and the other amino acids and micronutrients (vitamins, minerals, oligoelements and essential fatty acids in some of them) to prevent nutritional deficiencies.<sup>5-7</sup> An alternative treatment with BH<sub>4</sub> also succeeds in decreasing high Phe concentrations in patients who respond to this therapy.<sup>7,8</sup> Therefore, adverse clinical sequelae occur in patients with

Laboratory Center, Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China

LL YANG (楊莉麗) MSc

Department of Genetics and Metabolism, Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China

HQ MAO (毛華慶) BM

WF ZHANG (章偉芳) MPH

ZY ZHAO (趙正言) MD

RL YANG (楊茹萊) BM

XL ZHOU (周雪蓮) BM

XL HUANG (黃曉磊) MM

XW HUANG (黃新文) MD

Correspondence to: Dr XW HUANG

poor treatment compliance even after early diagnosis and treatment. PKU management remains challenging in many aspects including threshold levels of blood phenylalanine for starting treatment, target blood phenylalanine levels, and the management of older patient groups.<sup>2</sup>

PKU was the first newborn screening test introduced into China about three decades ago. The first PKU test was carried out in our newborn screening centre in 1999. In this study, we aimed to review the cognitive outcome of PKU patients diagnosed through the newborn screening program. The management and treatment procedures were also evaluated. Further improvements can be made based on the problems indicated by this study.

## Methods

This retrospective and descriptive study was carried out at Newborn Screening Center of Zhejiang Province, which is one of the largest newborn screening centres in China. This study was approved by the institutional review board of Children's Hospital, Zhejiang University School of Medicine.

### *Newborn Screening and Diagnosis of PKU*

Dried blood spots were collected on Whatman 903 filter paper between the third and fifth day of life from the babies' heels. All the newborn screening samples collected throughout the province were transported to the Laboratory Center at our hospital for analysis. The information on the filter paper of the newborns was entered into our newborn screening database. Blood phenylalanine concentration was measured with the fluorescent ninhydrine method (EG&G Wallac neonatal phenylalanine kit) using a fluorometric multitask plate counter Wallac 1420 VICTOR F (PerkinElmer, Finland). All procedures were performed according to the manufacturer's instructions. Quality control for PKU screening was provided by the Centers for Disease Control and Prevention (CDC) in the USA. The cut-off value for phenylalanine was 120  $\mu\text{mol/L}$ . All the newborns with phenylalanine concentrations  $>120 \mu\text{mol/L}$  were recalled for retesting. The nurses will contact the parents by telephones for recalling the newborns with abnormal results. Blood samples were taken for these children at fasting. Newborns were considered as positive when phenylalanine concentration  $>120 \mu\text{mol/L}$  at recall-testing. All the newborns with positive results were subjected to further diagnosis testing including urine analysis

for neopterin and biopterin and measurement of dihydropteridine reductase (DHPR) activity and  $\text{BH}_4$  loading test. Patients with  $\text{BH}_4$  deficiency were not included in this study.

### *Management and Follow-up*

PKU patients were divided into three groups by their birth year: group I, born from January 1999 to September 2001; group II, born from October 2001 to September 2008; and group III, born from October 2008 to September 2010. The dietary treatment consisted of a restricted protein diet supplemented with an essential amino acid mixture supplement. Doctors will carefully calculate the amount of breast milk or regular formula to be mixed with the phenylalanine-free formula for infants. Parents introduce solid foods with low levels of phenylalanine to children with PKU on the same schedule used for other infants. The status of PKU patients was closely monitored, and the dietary treatment protocol was adjusted according to the blood Phe concentration. Oral sapropterin dihydrochloride (10-20 mg/kg.day) was given to patients with tetrahydrobiopterin responsive PKU.

During the follow-up, Phe level is required to be tested every three days for those at the starting period of treatment, every two weeks for those less than one year of age, 1-2 times every month for 2-12 years of age and every three months for those older than 12 years of age. The reference values (National Institutes of Health 2000) for plasma phenylalanine control are 120 to 240  $\mu\text{mol/L}$  for children below 3 years old, 120 to 360  $\mu\text{mol/L}$  for those aged 3-9 years, 120-480  $\mu\text{mol/L}$  for those aged 9-12 years and 120-600  $\mu\text{mol/L}$  those over 12 years old. Gesell Developmental Schedule was used to evaluate neuropsychological development when children aged 1 year, 3 years and 6 years, respectively.

## Results

During the 11-year period, a total of 3,791,538 newborns had been screened for PKU in our centre. Totally 147 children (70 boys and 77 girls) were diagnosed with PKU after newborn screening with a prevalence of 1/25,600. Among them, four patients were found to be  $\text{BH}_4$  responsive. The mean DBS Phe level at newborn screening was 558.4  $\mu\text{mol/L}$  (range: 179.0-2219.1  $\mu\text{mol/L}$ ). The mean DBS Phe level at diagnosis was 1264.5  $\mu\text{mol/L}$  (range: 177.2-4211.4  $\mu\text{mol/L}$ ). The mean age at diagnosis for these

children was 37 days (range: 15-289 days). Among them, 67 children were referred to the centre and diagnosed at less than one month of age, and eighty older than one month of age delayed by their parents. Weight and height were measured in 49 children. Among them, 8 were found to be growth retarded compared to their peers. Four patients with BH<sub>4</sub> responsive PKU treated with sapropterin dihydrochloride had normal growth and development.

### Follow-up Information

After diagnosis, 24 children were refused to be treated by their parents. Four children were discontinued treatment after a period by their parents. Nine children were lost during the follow-up. Fourteen children were not treated at our centre, 8 of them were treated at children's hospital in Shanghai and 6 went back to their hometown hospitals. One child died several days after diagnosis due to non-PKU-related disease. Finally, only 96 children have been followed up and treated at our centre until now. Among them, thirteen patients were delayed to be treated at a mean age of 25 months (60 days-52 months).

### Outcome of the Patients

There were 8 children in group I, 53 in group II and 33 in group III.

Among the 8 children in group I, 3 had no complete data of the average Phe concentration in the first three years after birth. Of the three patients, one patient was treated at 52 months of age and one at 46 months of age. The reason was that their parents didn't believe in the diagnosis results

and refused treatment until the children became symptomatic. The two patients (2/6, 33.3%) had mental retardation with developmental quotient (DQ) at 45 and 60, respectively. The median level of annual mean concentration for the other 5 patients at the first three years after birth was 70.4, 297.4 and 475.3  $\mu\text{mol/L}$ , respectively. A majority of children (62.5%) in this group had poor treatment compliance with poor Phe concentration control (Table 1).

For the 53 children in group II, the median level of annual mean concentration for the patients at the first three years after birth was 261.30 (n=43), 302.91 (n=41) and 361.50  $\mu\text{mol/L}$  (n=31), respectively. Among of children, 47.5% had poor phenylalanine control. Management in this group of children was poor included: (1) delayed treatment in two patients (12 months and 8 months of age, respectively); (2) treatment was discontinued for a period and restarted after funding obtained in five patients; (3) very relaxed diet restriction or no regular clinical visits and laboratory testing in 17 patients. Eight of the 38 children (21.1%) in this age group who agreed for neuropsychological assessment had mental retardation (DQ: 31-69) (Table 1).

For 33 children in group III, all children had regular clinical tests results. According to the Phe concentration in the first three years, they had good Phe control (171.40  $\mu\text{mol/L}$ , 224.80  $\mu\text{mol/L}$  and 231.65  $\mu\text{mol/L}$ ). According to the point Phe concentration at the last follow-up visit, 19 (57.6%) children are below the reference level. One of 11 children (9.1%) had mental retardation (DQ: 63) (Table 1).

**Table 1** The follow-up data and outcome of the phenylketonuria patients

Variables	Group 1 (n=8)	Group 2 (n=54)	Group 3 (n=33)
The first year n, median Phe (range, $\mu\text{mol/L}$ )	5, 70.40 (37.00-291.00)	41, 261.30 (31.00-993.70)	33, 171.40 (10.70-1097.60)
Second year n, median Phe (range, $\mu\text{mol/L}$ )	5, 297.4 (58.4-881.7)	43, 302.91 (29.70-1066.40)	19, 224.80 (24.50-914.50)
Third year n, median Phe (range, $\mu\text{mol/L}$ )	5, 475.3 (165.3-966.6)	31, 361.50 (61.60-815.90)	2, 231.65 (130.40-332.90)
Phe level at the most recent clinic visit, median (range, $\mu\text{mol/L}$ )	528.15 (97.10-753.90)	465.60 (41.00-960.80)	297.2 (8.30-1454.70)
Phe concentrations meeting target ranges, n (%)	1 (12.5%)	23 (42.6%)	19 (57.6%)
Neuropsychological assessment			
Patients assessed, n	6	38	11
Evaluation results, n (%)			
Normal	4 (66.7)	30 (78.9)	10 (90.9)
Mental retardation	2 (33.3)	8 (21.1)	1 (9.1)

Phe: phenylalanine

## Discussion

The incidence of PKU varies widely in neonates around the world: it is high in Turkey (1:2600) and Ireland (1:4500) and low in Japan (1:125 000).<sup>9</sup> It was reported that the average prevalence of PKU in China is 1/11,572.<sup>10</sup> The prevalence in Zhejiang Province is much lower than the average level (1/25,600).

This eleven-year retrospective study revealed several problems in management PKU patients in our screening centre. Delays of referral, refusing treatment by parents, and noncompliance of treatment were found to be commonplace in the studied population. Delays of referral to the centre for diagnosis was common in the screened children. Vela-Amieva et al reported that the main cause of late referral of PKU patients was the absence of PKU screening.<sup>11</sup> Differing from them, the major reason for delays of referral in our patients is that China has a postpartum culture of "zuo yue zi" (sitting out the month). Mother and newborn stay indoors and are secluded for one month after birth.<sup>12</sup> For the children with positive screening results, nurses in our centre will contact their parents repeatedly and addressed the importance of early referral. However, most of them neglected our suggestion and insisted taking the child after the month of "zuo yue zi". Delays of referral after newborn screening due to this postpartum culture is a widespread problem in China. The other reason is that some parents do not believe in the testing results. Future education to the parents should be centred on the arms of delayed referral and diagnosis of children after newborn screening.

Twenty-four children never received any treatment after diagnosis in this study, 13 discontinued treatment or lost to follow-up. All these occurred before 2008. Yu et al also reported that 249 (41.2%) of 603 diagnosed PKU children in a hospital in Beijing refused or discontinued treatment by the parents.<sup>13</sup> In our study, majority of the parents who refused or discontinued treatment for children were migrant workers of low education level or living in rural areas. The high cost of the PKU treatment is a major problem. Treatment refusal rarely occurred after our centre applied a PKU funding for supporting the family from low social and economical level since 2008. The patients' Phe control also got improvement and the percentage of patients with mental retardation reduced since 2008. Refusing or discontinuing treatment are rarely reported in the countries with medical insurance coverage for children.<sup>11,14</sup> Unlike full or partial reimbursement in many developed countries, the cost for newborn screening has to be self-paid in China.

Contrary to the high rate of treatment refusal in Yu's<sup>13</sup> and our centre, only 3 out of 96 children refused treatment due to economical burden reported by Liu et al<sup>15</sup> in Xuzhou City. The hospital pays half the cost of medication for all PKU patients, and all the cost of laboratory testing are free there. Therefore, medical insurance coverage or economical support to the PKU family will improve the treatment compliance.

Delay or no treatment will lead to mental retardation in PKU patients.<sup>11</sup> It was reported that 84-98% of untreated PKU patients had IQ lower than 50.<sup>16</sup> The 24 children untreated and the 9 lost to follow-up in our centre may have developed mental retardation later. González et al reported that 97.7% (diagnosis <2 months of age) of the early diagnosed patients had normal IQ, while 46.3% of late diagnosed patients (>2 months of age) had mental retardation.<sup>17</sup> For our children, almost all the children were diagnosed as early as less than two months of age. However, early diagnosis didn't represent early treatment in our children. Among the eleven children with mental retardation, 8 were diagnosed at <2 months of age but were treated very late at a mean age of 25 months. 61.5% of the late treated children had mental retardation in this series. Therefore, early diagnosis and treatment are crucial for optimal neuropsychological outcome in PKU patients.

Poor compliance of treatment remains a big challenge in PKU management. Significant noncompliance exists in PKU patients. Playle and Keeley reported 25 to 50% of patients might vary from a phase of compliance to one of noncompliance at some time.<sup>18</sup> Noncompliance of the treatment leads to not well-controlled Phe levels in all the age groups. Blood Phe concentration may be well controlled in the first few years, then deteriorating with age.<sup>19,20</sup> In our studied children, Phe concentration also increased with age during the first three years after birth. The percentage of patients with Phe concentration meeting target range decreased with age. In the group aged 9-12, only 12.5% of the patients had normal Phe concentration. The percentages of blood Phe concentrations meeting target ranges were much higher in other reports (88% in children aged up to 1 year, 74% for 1-10 years, 89% for 11-16 years).<sup>19</sup> The underlying reasons of non-compliance in our patients include poor access to treatment due to economical problem, illiteracy of parents, poor knowledge/insight into the disease, disinterest on children's disease, disbeliefs about the nature of the disorder and resistance of treatment, low educational status to valid information, children living with grandparents and so on. Except the above mentioned

factors, our screening mode may have affected the treatment compliance implicated in this study. For the family living far from the hospital, it takes the parents time and money on travelling to take a follow-up visit. We are now attempting to establish a follow-up network via internet, and carry out the routine training program for the pediatricians from the local hospitals. More patients will be followed up at local hospitals or by remote consultation in near future.

This study has several limitations. We underestimated the importance of the timely neuropsychological assessment during the early period of screening. Our guideline also does not require strict neuropsychological assessment at 1, 3 and 6 years of age before 2008. Therefore, the data presented were incomplete, and the dropout rate for treatment among the PKU patients is high. Also not all the treated patients received cognitive assessment. The number of patients with mental retardation may be increased if all children were assessed.

In conclusion, economical burden affects the management and follow-up of the patients a lot. The outcome of the late diagnosed and treated children was poor. Late treatment will lead to severe mental retardation. Non-compliance to the treatment was common in the studied children. The Phe concentration control may become more difficult when these children go into their adolescence. Urgent measures have to be done to improve the screening strategies for improving the treatment compliance and making screening more cost-effective. On one hand, we will promote public education on the importance of newborn screening via social media and internet. On the other hand, we are calling for the policymakers to include newborn screening into the basic health insurance for children. Funding may take a key role on solving the problem of delayed treatment and poor compliance. Social and community support should as well be strengthened, and education programs for parents will be implemented in the screening collaborative hospitals and clinics as soon as possible.

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