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Stability of blood phenylalanine levels and IQ in children with phenylketonuria

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ABSTRACT

Variability of metabolic control in phenylketonuria (PKU) potentially affects cognitive outcome in early and continuously treated children with this condition. The possibility that homeostasis is more important than the absolute level of exposure to phenylalanine (phe) has not previously been examined. A meta-analysis of 40 studies showed that in children with phenylketonuria (PKU), mean lifetime blood phe levels were significantly correlated with Full Scale IQ (FSIQ) ($r = -0.34$). A similar correlation ($r = -0.35$) was found between FSIQ and mean exposure during 0–12 years of age. Most of the studies in the meta-analysis, however, included children who had discontinued the phe restricted diet. None examined the impact of fluctuations in metabolic control in continuously treated children. This is important because new therapies may increase stability in blood phe levels. The question has arisen whether these therapies are beneficial in children whose blood phe levels are generally within the recommended range of 120–360 μmol/L. In this study, we describe the relationship between FSIQ and two parameters of metabolic control: (1) mean blood phe level of all reported specimens for each subject, and (2) variability of the blood phe level as indicated by the standard deviation of blood phe levels for each subject. Analyses were performed using lifetime phe levels and levels during three periods (0–6 years, 0–10 years, and >10 years of age). The most recent FSIQ for each child was used in the correlation analyses.

Data were collected from medical records on all 46 children born between 1999 and 2006 with early and continuously treated PKU followed at the Metabolism Program at Children's Hospital Boston. The mean age of the children at the time of their most recent FSIQ test was 7.5 + 3.3 (2.9–15.5) and their mean FSIQ was 104 + 15 (68–143). The mean lifetime blood phe level in these children was 312 + 132 μmol/L (125–852). The standard deviation of blood phe levels was 182 + 72 μmol/L (96–336). The correlation between lifetime blood phe levels and most recent FSIQ was −.17 ($p = 0.38$) and the correlation between standard deviation of blood phe levels and most recent FSIQ was −.36 ($p = .058$), not reaching significance, but indicating a trend. These results indicate that stability of blood phe levels may be more important to cognitive functioning than overall exposure to phe in early and continuously treated PKU. In treating PKU, attention should be given to variability in blood phe levels as well as maintenance of phe levels within the recommended range.

Introduction

Phenylketonuria (PKU) is an autosomal, recessive disorder characterized by the defective hydroxylation of phenylalanine (phe), an essential amino acid found in all protein. Due to the absence or deficiency of the liver enzyme, phenylalanine hydroxylase, phe cannot be converted into tyrosine, resulting in both the toxic accrual of phe and a deficiency of tyrosine [1]. PKU can be divided into four categories based on severity. We define classic PKU as natural blood phe levels greater than 1200 μmol/L. Moderate PKU is characterized by natural blood phe levels between 900 and 1200 μmol/L. Mild PKU is characterized by natural blood phe levels of 600–1199 μmol/L. Blood phe levels of 120–599 μmol/L signify mild hyperphenylalaninemia which usually does not require treatment.

With the initiation of newborn screening programs in the 1960's, early detection and treatment of PKU became possible, nearly eliminating the risk of neurological consequences traditionally associated with untreated PKU, including mental retardation, schizophrenia, self-abuse and decreased coordination [2]. The only known cure for PKU is liver transplantation [3]. Given the risk inherent in this option, almost all children with PKU are treated via dietary treatment.

Worldwide consensus regarding blood phe levels necessary to achieve optimal development in PKU does not exist. Nonetheless, the majority of clinics in the United States follow the recommenda-

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tion of the NIH Consensus Conference to maintain blood phe levels between 120 and 360 μmol/L [4]. This can only be achieved through strict adherence to a low-phe dietary regimen, including avoidance of meat, fish, dairy products, nuts, beans and other foods that are comprised of proteins. In addition, a supplemental formula rich in all other essential amino acids must be consumed to provide adequate protein intake [5]. Since many children experience difficulties adhering to the diet and tolerating the strong flavor of the formula, ideal blood phe levels are often not achieved [6].

Bickel et al. first described the effectiveness of treatment in improving the intellectual development of children with PKU [7]. Years later, the International PKU Collaborative Study reported that continuing dietary treatment with recommended blood phe levels below 600 μmol/L until age 8 years resulted in Full Scale IQ (FSIQ) similar to that of siblings and parents [8]. Recently, however, researchers demonstrated benefits of more restrictive dietary treatment throughout life. Strict dietary control (blood phe <360 μmol/L) reduced the incidence of impaired cognitive functioning, diminished school achievement, and problem behaviors in continuously treated patients with PKU [9]. A recent meta-analysis showed that in children with PKU, mean lifetime blood phe levels were significantly correlated with FSIQ ($r = -0.34$) [10]. A similar correlation ($r = -0.35$) was found between FSIQ and mean exposure during 0–12 years of age. Most of the studies in the meta-analysis, however, included children who had discontinued the phe restricted diet. None examined the impact of fluctuations in metabolic control in continuously treated children. This is important because new therapies may increase stability in blood phe levels. These include supplementation with tetrahydrobiopterin (BH_4), which is a co-factor for the phenylalanine hydroxylase enzyme [11] and potential enzyme replacement therapy [12, 13]. The question has arisen whether these therapies are beneficial in children whose blood phe levels are generally within the recommended range of 120–360 μmol/L.

In this study, we describe the relationship between FSIQ and two parameters of metabolic control: (1) mean blood phe levels of all reported specimens for each subject, and (2) variability of blood phe levels as indicated by the standard deviation of blood phe levels for each subject. The objective is to examine the question: is stability of blood phe related to cognitive outcome in early and continuously treated PKU?

Methods

A retrospective chart review of the neuropsychological records maintained by the Metabolism Program at Children's Hospital Boston provided data on developmental functioning, intelligence, phe levels and genotype. These children were seen in the Metabolism Clinic, had early and continuously treated PKU, were born between 1991 and 2006, had at least two phe levels recorded and at least one FSIQ or Developmental Quotient (DQ) score. Children with mild hyperphenylalaninemia who did not receive treatment were excluded from the study.

Most children seen at the metabolism clinic are routinely administered developmental testing at 6, 12 18 and 24 months of age. Neurocognitive testing is performed at 4, 5, 7, 10, 13 and 16 years of age. When a child is having difficulties, more frequent evaluations are conducted.

The Mental Development Index (MDI) from the Bayley Scales of Infant Development (BSID, BSID-II-52 tests) and or the Cognitive score (BSID-III-5 tests) was used to measure developmental function in children younger than age 40 months. IQ scores of children 40 months and over were obtained from various tests, depending on the age of the child. These included: the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-R-13 tests, WPSSI-III-12 tests), Wechsler Intelligence Scale for Children (WISC-III-19 tests, WISC-IV-8 tests), and Wechsler Abbreviated Scale of Intelligence (WASI-11 tests). The General Cognitive Index (GCI) of the McCarthy Scales of Children's Abilities was also used (26 tests) since the GCI is considered comparable to a FSIQ, an assumption supported by the reported correlation of .68 between the McCarthy GCI and the WPPSI-R FSIQ [14]. The Committee on Clinical Investigations at Children's Hospital Boston approved the chart review.

Descriptive statistics were obtained for data related to blood phe and neurocognitive assessments. Descriptive statistics are reported on all blood phe level determinations, but only blood phe levels obtained up to the time of the last (most recent) intelligence test were included in the correlational analyses of blood phe and IQ. Each child was classified as having mild, moderate or classic PKU based on genotype and phe tolerance. Each child's blood phe levels were plotted against age at the time of obtaining the specimen. These plots were visually inspected to obtain a sense of the patterns of variability. The number of spikes, defined as a change from the previous or subsequent phe level of greater than 600 μmol/L, was also observed. In addition, age was correlated with blood phe level. The similarity of the Developmental Quotient (MDI) and FSIQ was analyzed with a paired t-test to determine if results in our sample differed from published norms [15]. The effect of age on MDI was estimated using each MDI measure for each subject and using a generalized estimating equations (GEE) regression model to account for correlations among the repeated observations on a subject [16]. The effect of age on FSIQ was similarly estimated. Because no significant difference between MDI and FSIQ scores was noted, the most recent FSIQ score was used for the two primary analyses. Pearson correlation statistics were computed to test for association between (1) mean lifetime blood phe levels and most recent FSIQ, and (2) standard deviation of lifetime blood phe levels and most recent FSIQ. For each of these analyses, there was only one observation per subject. Linear regression was used to estimate the size and significance of the effects of mean and standard deviation of phe levels on FSIQ. Secondary regression analyses included age, mean and standard deviation of blood phe levels during critical periods (under 6 years of age; under 10 years; and 10 years and older) as potentially significant covariates.

Results

Among the 46 children in the study, 26 (56%) had genotypes associated with classic PKU, while 15 (33%) were classified as having moderate, 4 (9%) with mild and 1(2%) with unclassified PKU (genotype not associated with a specific phenotype). As shown in Table 1, the patients included in the study received careful follow-up, with frequent monitoring of blood phe levels and neurocognitive functioning. The mean blood phe level for the group was based on all of the phe levels recorded (the mean of the subjects' means), and was well within the recommended range of 120–360 μmol/L. The standard deviation of lifetime blood phe levels (mean of the subjects' standard deviations) reflects the finding that out of 45 child with greater than 2 phe levels, 21 (47%) had at least one spike in blood phe level of greater than 600 μmol/L (see Figs. 1 and 2). The FSIQ among these children ranged from 68 to 143. Of the 46 children in the study 45 had more than 10 phe levels recorded. Blood phenylalanine levels in children followed at our clinic are monitored every week during the first 3 months of life and every month thereafter. Compliance with this protocol is variable in some patients. The mean developmental score (DQ) of the entire group based on all DQ scores and the mean FSIQ based on all FSIQ scores of the entire group were well within the average range. The correlation between the DQ and FSIQ was significant (rho = .50; $p = .007$). A paired t-test comparing the DQ and FSIQ

Table 1
Description of the sample

Total sample size (23 boys and 23 girls)	46
Mean age ± SD (range) at final intelligence testing	7.5 ± 3.32 years (2.92–15.5)
Total number of blood phe determinations in database	4751
Mean number ± SD (range) of blood phe determinations per child	103 ± 74.9 (1–254)
Mean phe ± SD (range) of the group based on all phe levels (mean of the subjects' means)	312 ± 132 μmol/L (125–852)
Mean standard deviation (range) ± SD of lifetime blood phe levels (mean of the subjects' standard deviation)	182 ± 72 μmol/L (96–336)
Total number of IQ test scores in the database (n = 32 subjects)	89
Total number of DQ test scores in the database (n = 28 subjects)	87
Mean DQ ± SD (range) of the group based on all DQ scores	103 ± 12 (77–131)
Mean IQ ± SD (range) of the group based on all IQ scores	102 ± 14 (68–143)
Mean IQ ± SD (range) of the group based on most recent IQ	104 ± 15 (68–143)

phe = phenylalanine, SD = standard deviation.

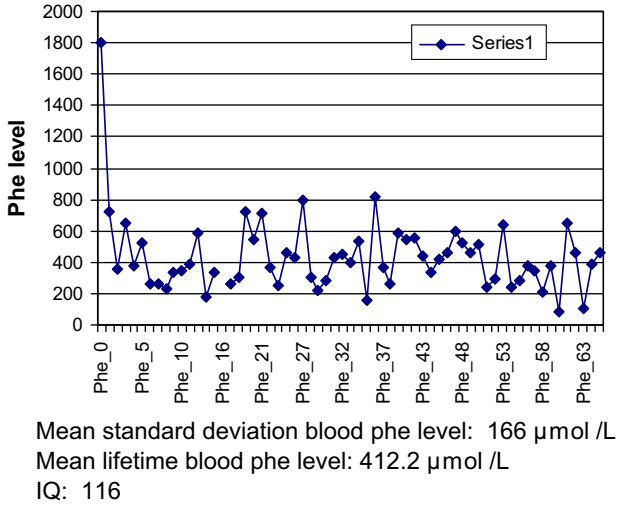

Fig. 1. Example of low standard deviation blood phe (7-year 6-month-old female with classic PKU).

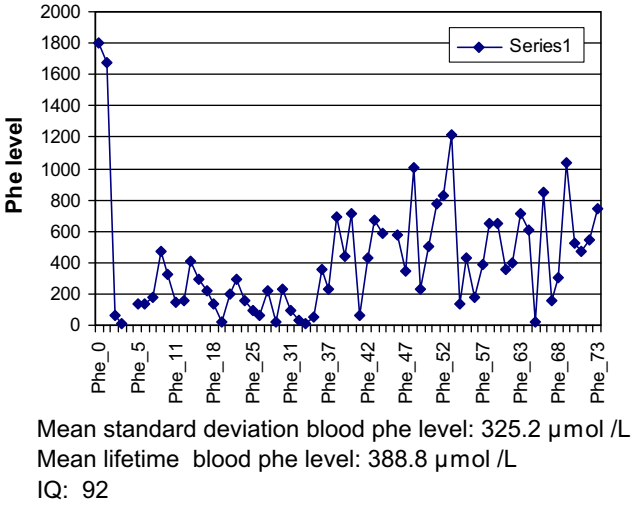

Fig. 2. Example of high standard deviation blood phe (6-year 1-month-old male with classic PKU).

Table 2
Critical periods: mean lifetime blood phe and mean SD blood phe levels and Pearson correlations with Full Scale IQ*

Age range in years (n)	Mean lifetime phe ± SD (μmol /L) (range)	Correlation of mean lifetime phe with most recent FSIQ (p)	Mean SD phe ± SD (μmol /L) (range)	Correlation of mean SD phe and most recent FSIQ (p)
0–<6 (27)	305 ± 92 (115–490)	−.24 (p = .24)	176 ± 76 (76–326)	−.30 (p = .14)
0–<10 (32)	334 ± 140 (125–852)	−.18 (p = .32)	173 ± 70 (83–325)	−.36 (p = .05)
>10 (15)	520 ± 308 (164–1130)	−.16 (p = .57)	210 ± 205 (83–900)	−.45 (p = .11)

FSIQ = Full Scale IQ, phe = phenylalanine, SD = standard deviation.
* Includes only children who had a Full Scale IQ score (excludes those with only Developmental Quotients).

scores showed that the difference in the scores was not significant (t_{27} = 1.24, p = 0.23). IQ and age at testing were not correlated (rho = −.025, p = .89).

The correlation between the standard deviation of blood phe levels and most recent FSIQ was −0.36 (p = .06). In a regression model, FSIQ decreased 4.3 points with every 1 point increase in standard deviation of blood phe. Over the observed range of standard deviation in phe levels, the predicted decrease in FSIQ was 18 points.

The correlation between lifetime blood phe and most recent FSIQ was not significant (r = − 0.17, p = .38). In a regression model testing the combined effects of mean blood phe level and standard deviation of blood phe on FSIQ, the estimated effect of the standard deviation of blood phe was similar in magnitude to that reported in the univariate model above (β = −5.5, p = 0.07). The effect of blood phe level was smaller (β = 1.1, p = 0.56) and in a slightly positive direction, with higher phe associated with higher FSIQ. The standardized betas for mean and standard deviation of phe level were 0.14 and −0.45, respectively.

Mean blood phe levels and mean standard deviation blood phe levels were significantly correlated (r = 0.51, p = .004), indicating that children with higher blood phe levels also experienced greater variability.

As noted in Table 2, mean blood levels and the standard deviation of blood levels rose slightly at each "critical" period. Neither the mean blood phe level nor the standard deviation blood phe level was correlated with FSIQ at any of the critical periods, although the correlation between standard deviation blood phe level and FSIQ was higher than the correlation between mean blood phe and FSIQ at each critical period with p values between 0.05 and 0.14.

Discussion

This study demonstrates that variability of blood phe levels is more closely related to cognitive outcome than the mean lifetime blood phe level in early and continuously treated children with PKU. These results may not apply to children whose blood phe levels are generally above the recommended range, although variability may exacerbate the effect of elevated phe exposure. Since the mean age of our sample was 7 years old, it is possible that variability may not be as critical a factor in older children. However, our analyses suggest that in the small number of children over age 10 years, variability in phe exposure continued to be more important than the blood phe level itself in explaining cognitive scores.

Genotype may play a role in the variability of phe levels in children with PKU. In our study, the 10 children with the highest standard deviation scores had genotypes corresponding to classic PKU. Of the 10 children with the lowest standard deviation scores, 2 had

classic PKU, 5 had moderate, 2 had mild PKU and 1 had unclassified PKU. This is consistent with the fact that children with more moderate forms of PKU tolerate greater levels of phe intake than children with classic PKU, who need a more stringent diet to maintain blood phe levels in the recommended range [17] and possibly to maintain stability in their blood phe levels.

Metabolic control can not be characterized solely on blood phe levels since tyrosine levels and tyrosine:phe ratios are also important [18]. Moreover, when considering cognitive outcome, brain phe levels may have a greater impact on functioning than blood phe levels, and these two parameters are not interchangeable [19].

Treatment strategies that enhance stability of the blood phe level should seriously be considered, even for children who adhere to current treatment recommendations. This means that new therapies that are now available and others that may be instituted in the near future may contribute to optimal outcome in PKU. Occasional "spikes" in blood phe levels occur in the majority of children with PKU. The results of this study suggest that such variations may be benign and that it is long-term variability that may be important even in a child who has one or two of these "spikes" or drops of approximately 600 μmol/L. In conclusion, more attention should be given to variability in blood phe levels as well as to maintenance within the recommended range.

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