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Maternal Hyperphenylalaninemia: Rapid achievement of metabolic control predicts overall control throughout pregnancy

Teresa Martino^a, Celide Koerner^a, Gayane Yenokyan^b, Julie Hoover-Fong^c, and Ada Hamosh^a

Teresa Martino: tmarti34@jhmi.edu; Celide Koerner: ckoerne1@jhmi.edu; Gayane Yenokyan: gyenokya@jhsph.edu; Julie Hoover-Fong: jhoover2@jhmi.edu; Ada Hamosh: ahamosh@jhmi.edu

^aMcKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Blalock 1008, Baltimore, MD 21287, USA, Phone: 1.443.287.4588, Fax: 1.410.614.9246

^bJohns Hopkins Biostatistics Center, Bloomberg School of Public Health, 615 North Wolfe Street, Room E3153, Baltimore, MD, 21205, USA

^cMcKusick-Nathans Institute of Genetic Medicine, Greenberg Center for Skeletal Dysplasias, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287, USA

1. Introduction

Hyperphenylalaninemia (HPA) is an autosomal recessive, inborn error of metabolism, typically characterized by deficient or absent activity of the enzyme phenylalanine hydroxylase (PAH), (in rare cases there is impaired recycling of tetrahydrobiopterin (BH4)) [1]. Although the incidence of HPA is 1 in 10,000 among Caucasians [2], there is a markedly higher incidence in other ethnicities, such as Irish (1 in 4,500) and Turkish (1 in 2,600) populations [3,4]. In individuals for whom treatment for HPA is inadequate, delayed, or who are not treated at all, there is a risk for the development of serious behavioral and psychiatric problems [5].

To maintain metabolic control, the patient typically is supported by a diverse care team comprising a biochemical geneticist, a nutritionist, and family. Frequent monitoring of the plasma phenylalanine (Phe) concentration is essential to adjust Phe intake, and monitor metabolic control. A Phe-restricted diet is difficult to follow, and poor dietary compliance is common in HPA, especially in adolescence when social factors are influential [6]. Adults may also experience difficulty maintaining dietary compliance especially if they have been exposed to diverse, more palatable regular foods. In the United States, a variety of low-Phe medical foods are produced. They are often particularly expensive, relatively inconvenient to use and have limited availability, in addition to often being less palatable than foods that are not restricted in Phe. Currently, those with milder forms of HPA may achieve metabolic control with a combination of dietary therapy and medical treatment using sapropterin (BH4) [7].

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Inadequate adherence to a low-Phe diet is particularly detrimental to pregnant women of childbearing age, as elevated Phe in pregnancy is a form of metabolic teratogenesis [8]. Maternal Hyperphenylalaninemia (MHPA) is the term used to define women with HPA who are pregnant. Very poorly controlled MHPA results in neonatal microcephaly (73%), mental retardation (92%), congenital heart defects (12%), and intrauterine growth restriction (40%) [9]. Facial dysmorphism also occurs, including a wide, flat nasal bridge, wide outer canthal distance, long palpebral fissures, a smooth philtrum, anteverted nares, and low set, posteriorly rotated ears [10]. Elevated maternal plasma Phe is of special concern during critical periods of embryogenesis [11].

Adherence to a Phe-restricted diet is critical in pregnancy, and ideally prior to conception. To prevent poor outcomes in the children of women with MHPA, affected pregnant women and their providers aim to control the plasma Phe concentration throughout pregnancy. The desired target range for the plasma Phe concentration is 120–360 $\mu\text{mol/L}$, as established by the Maternal Phenylketonuria Collaborative Study (MPKUCS) [12]. At our institution, we aim for an upper threshold of 300 $\mu\text{mol/L}$.

While elevated plasma Phe results in teratogenesis, sub-physiologic plasma Phe may indicate protein deficiency in the pregnant patient. The latter paradoxically results in elevation of plasma Phe, due to muscle catabolism to compensate for a low plasma Phe concentration. Catabolism also occurs with insufficient calorie intake. Therefore, a delicate balance must be maintained between providing an adequate amount of calories and synthetic protein (Phe-free protein) to meet the needs of the pregnant patient and her fetus, while limiting Phe intake to permit metabolic control.

While it is known that maintaining maternal plasma Phe levels in the target range prior to conception and throughout pregnancy reduces the incidence of teratogenic effects in the offspring of women with MHPA [8,10,11,12,13], rigorous studies of the optimal and precise treatment methods to achieve such metabolic control are still needed. At our institution, a major component of the medical management of MHPA consists of weekly visits to the genetic metabolic nutritionist. While dietary compliance is essential to achieving metabolic Phe control, the intervention of the genetic metabolic nutritionist in the dietary management of MHPA is an integral part of successful day-to-day Phe management in pregnancy.

To test our institutional guidelines of managing MHPA, we undertook a retrospective study analyzing the effect of achieving metabolic control and maintenance in MHPA using 18 years of data from the Johns Hopkins Biochemical Genetics Clinic. Usual care consisted of weekly visits to a genetic metabolic nutritionist, with a dietary review, calorie count, weight check and Phe intake calculation. Dietary changes were implemented based on the adequacy of metabolic control determined by the fasting plasma Phe concentration on the day of the visit and interval weight gain. We hypothesized that women with MHPA who achieved metabolic control soon after their first encounter with the genetic metabolic nutritionist were more likely to maintain control throughout the remainder of the pregnancy than were women who did not achieve control soon after their first encounter with the metabolic nutritionist.

2. Materials and Methods

2.1 Patient population

The Institutional Review Board at Johns Hopkins approved the study. Cases for our retrospective analysis were identified by a record query of women with MHPA who received care between January 1991 and October 2009. Forty-seven pregnant women with MHPA were identified who received treatment at the Johns Hopkins University McKusick-Nathans Institute of Genetic Medicine. From review of maternal medical records, we

extracted data on demographics, gravidity, parity, gestational age at delivery, pregnancy outcome, HPA class (severity of HPA), plasma Phe concentration drawn at each visit to the genetic metabolic nutritionist, and gestational ages at these visits. Data used in the study were entered into a secure, encrypted database.

2.2 Plasma Amino Acid Analysis

Plasma amino acid concentrations were measured by standard ion exchange chromatography procedures using a Beckman 6300, Biochrom 20+, or Biochrom 30 amino acid analyzer [14].

2.3 Patient Classification

The women were classified by their degree of HPA severity before pregnancy, as determined by the plasma Phe concentration when on an unrestricted diet (most often, the original diagnostic plasma Phe concentration). At our institution, we defined 3 classes of HPA: (i) classical phenylketonuria (CP), plasma Phe concentration $>1,200 \mu\text{mol/L}$; (ii) variant phenylketonuria (VP), plasma Phe concentration between $400\text{--}1,199 \mu\text{mol/L}$; and (iii) benign HPA (BH), plasma Phe concentration $<400 \mu\text{mol/L}$. The plasma Phe concentration in HPA conditions is considered optimal when in the range of $120\text{--}360 \mu\text{mol/L}$.

We grouped the MHPA patients by their plasma Phe concentration at their first and second encounter with the nutritionist. We assessed the predictive value of achieving metabolic control by the second encounter on the ability to sustain metabolic control for the remainder of the pregnancy. The first encounter was defined as the first visit to the nutritionist during the pregnancy. The second encounter with the nutritionist typically occurred 6–7 days after the first encounter, a time span which allowed for physiologic correction of an elevated plasma Phe concentration. Some women were in very poor control at the first encounter, prompting either admission to the hospital with daily plasma Phe concentration measurements, or increased frequency of outpatient visits with the plasma Phe concentration drawn at each visit, to achieve metabolic control. In these cases, the second encounter was still defined as the plasma Phe concentration obtained 6–7 days after the first encounter. We defined plasma Phe control as either in the target (IT) range of $120\text{--}360 \mu\text{mol/L}$, or above the target (AT) range or $>360 \mu\text{mol/L}$ at the time of the second encounter (Figure 1).

2.4 Dietary management during pregnancy

Although women and adolescents with HPA conditions followed in our clinic were strongly encouraged to seek counsel from our genetic metabolic nutritionist to achieve optimum metabolic control prior to conception, most came to us already pregnant. Once pregnant, they were expected to visit the nutritionist weekly. Throughout their pregnancies, they were asked to keep a daily dietary record of all foods and beverages; and from this we analyzed the Phe, synthetic protein, calorie and fluid intake. On a weekly basis, the genetic metabolic nutritionist reviewed these data, as well as information on maternal weight, blood pressure, and the plasma amino acid analysis.

In pregnancies of women with CP or VP, the synthetic protein prescription remained constant, and caloric intake was adjusted based on the rate of maternal weight gain. At baseline, pregnant women with CP or VP were prescribed 0.8 gm/kg/day of synthetic protein (a blend of essential and non-essential amino acids without Phe, and fortified with additional tyrosine) plus an additional 10 grams of synthetic protein to support fetal growth, maternal weight gain, and to maintain maternal protein storage. The total caloric intake was set at 35 cal/kg/day . The prescribed Phe intake quantity varied from individual to individual based upon the severity of HPA, the residual PAH enzyme function, maternal weight, inter-

current illness, stressors, and gestational age. The plasma Phe concentration was monitored weekly.

Pregnant women with BH followed a different dietary regimen. Their diet prescription provided 0.8 gm/kg/day of natural protein derived from regular foods plus an additional 10 grams of natural protein to support fetal growth, maternal weight gain, and to maintain maternal protein storage. Their dietary intake was closely monitored for intake of natural protein, rather than Phe, with natural protein derived from a typical diet rather than from synthetic protein. Occasionally, the prescribed level of natural protein intake resulted in plasma a Phe concentration that exceeded 360 $\mu\text{mol/L}$. In this rare situation, MHPA patients with BH were supplemented with synthetic protein; however, their natural protein intake was maintained at the highest amount tolerated. Pregnant women with BH were prescribed a total daily caloric intake of 35 cal/kg/day that was adjusted throughout pregnancy. The plasma Phe concentration was monitored weekly.

Although our retrospective data contain information on the treatment of two pregnant women with BH, they were excluded from the statistical analyses, because they were encouraged to consume natural protein, as opposed to complete restriction of natural protein in CP and VP. Furthermore, their diagnostic Phe concentration used to determine the HPA severity classification was based on a plasma Phe concentration of $<400 \mu\text{mol/L}$ while on a regular diet. This value is only slightly above the upper limit of the target range of 120–360 $\mu\text{mol/L}$. Thus, pregnant women with BH, in general, do not have as much difficulty in achieving metabolic control as do those with CP or VP. MHPA patients with BH often have a plasma Phe concentration within the target treatment range, whether Phe intake is restricted or not. Using a slightly higher target range cut-off, Levy et al. has demonstrated milder forms of HPA are less teratogenic than more severe forms of HPA [15,16].

2.5 Phenylalanine Tolerance

At each genetic metabolic nutritionist visit during pregnancy, patients were prescribed an acceptable level of daily Phe intake calculated using the fasting plasma Phe concentration obtained on the same day. The plasma Phe concentration measured on the day of the visit was used as a benchmark for increasing, decreasing or maintaining the current daily Phe intake. Phe tolerance is defined as the daily Phe intake necessary to maintain the plasma Phe concentration in the target range.

2.6 Statistical Analysis

Descriptive analysis of demographic features was performed with calculation of means and standard deviations for continuous variables, and proportions for categorical variables. Comparison across Phe control groups was performed using Student's t-tests or Wilcoxon rank sum tests for continuous variables and chi-square or Fisher's exact test for categorical variables as appropriate. Multiple linear regression was used to determine the relationship of the average plasma Phe concentration for the remainder of pregnancy after the second encounter to the plasma Phe concentration at the time of the first encounter and second encounter. Regression coefficients are presented with 95% confidence intervals (CI). Using weekly Phe data, the average plasma Phe concentration was modeled as a function of gestational age, Phe control at the second encounter and other pregnancy-related and maternal characteristics. The association between average plasma Phe concentration and continuous predictors (gestational age, gravidity, parity, and maternal age) were explored visually using scatterplots and lowess smoothers. Linear splines with appropriate knots were added to reflect deviations from linearity. Linear mixed effects models with random intercepts for pregnancy were used to account for the hierarchical structure of the data, for example, visits nested within pregnancy. Several additional extensions to the model

described above were considered: a) a model with 2 random intercepts: for mother and for pregnancy, which were assumed independent and normally distributed with respective variances; b) a random slope at the pregnancy level to allow for heterogeneity in plasma Phe concentration trajectories with gestational age across pregnancies; c) an interaction of gestational age with plasma Phe control at the second encounter. The models were compared using AIC and likelihood ratio tests, and the most parsimonious model was chosen. Phe tolerance levels were compared by HPA class and trimester. Ratios of Phe tolerance were estimated using a generalized linear model with natural logarithm link function taking into account clustering by pregnancy. Statistical analyses were performed using STATA software (version 12.0, StataCorp LP, College Station, TX).

3. Results

For the analysis we included MHPA patients with CP or VP who had a term or preterm delivery. Twelve pregnancies were excluded from the study due to miscarriages (n=4) or terminations (n=4), BH (n=2), loss to follow up (n=2), and initiation of pharmacologic treatment of MHPA (n=1). Twenty women representing 35 pregnancies were available for analysis (Table 1). Maternal ages ranged from 22 – 39 years. At the time of the second encounter, there were 25 patients in the IT group, and 10 in the AT group. There were no differences between the two groups in maternal age, race, gestational age at delivery, and HPA severity class (Table 1). Of note, there was a statistically significant difference in gravidity, parity and gestational age at the first encounter where those in the IT group were less gravid, less parous and had their first encounter at an earlier gestational age (Table 1).

There was no significant difference in the mean plasma Phe concentration during pregnancy, according to HPA severity, after adjusting for pregnancy clustering. Mothers with CP had a higher average total plasma Phe concentration of 21 $\mu\text{mol/L}$, but this difference was not statistically significant (p-value 0.5; 95% CI: –33, 75). After adjusting for the plasma Phe concentration at the first encounter, the plasma Phe concentration at the time of the second encounter was on average 293 $\mu\text{mol/L}$ lower than the AT group (p-value: <0.001; 95% CI: –386, –200).

The individual weekly Phe concentrations throughout pregnancy were on average 78 $\mu\text{mol/L}$ lower in the IT group than the AT group at the second encounter, after adjusting for the metabolic control status at the first encounter (p-value: 0.008; 95% CI: –137, –20). Following this trend, the average plasma Phe concentration for the remainder of the pregnancy after adjusting for control status at the first encounter was 72 $\mu\text{mol/L}$ lower in the IT group as compared to the AT group (p-value: 0.02; 95% CI: –133, –10).

After the second encounter, MHPA patients in the IT group appear to have different trajectories of plasma Phe concentrations during their pregnancies, as compared to those who were in the AT group at the second encounter (Figure 2). Similarly, in a model which adjusted for date of second encounter, birth cohort and gravidity, the plasma Phe concentrations declined until the third trimester; however, the decline is less pronounced in the IT group than the AT group (–8.2 $\mu\text{mol/L/week}$ for IT [95% CI: –11.8 to –4.6], and –15.7 $\mu\text{mol/L/week}$ for AT [95% CI: –21.9 to –9.5]; p-value for interaction: 0.04).

Because of the difference noted between the IT and AT groups with regard to parity, a separate analysis was performed to determine the association between the plasma Phe concentrations of MHPA women and parity. In comparing pregnant women with one child and those with no previous children, there was no statistically significant difference in the individual plasma Phe concentrations after accounting for pregnancy clustering. However, there was more scatter of higher plasma Phe concentrations throughout pregnancy in women with one child (Figure 3). Moreover, from a multiple mixed effects regression adjusting for

the gestational age at the first encounter, gravidity, parity, and trajectories, there was no significant difference in the plasma Phe concentrations of the pregnancies according to maternal birth year cohort when categorizing women born earlier than 1965 as compared to those born from 1965–1975 and those born later than 1975.

The Phe tolerance was evaluated for 26 women pregnancies of 16 women for whom these data were available. The data were analyzed by trimester and by severity of HPA. Throughout each trimester, the Phe tolerance for VP was considerably higher than for CP (Table 2). When the Phe tolerance was evaluated for HPA severity alone, VP and CP both had a statistically significant trend toward higher Phe tolerance comparing the second to the first trimester, the third to second trimester and the third to the first trimester (Table 2).

Discussion

This study demonstrates that achieving plasma Phe control in MHPA by the second nutritional visit is a strong predictor of continued control throughout the remainder of the pregnancy. The visit is used for education and guidance of the patient, which are important components in establishing and maintaining Phe control throughout pregnancy. Prior to conception, women with HPA considering pregnancy should be encouraged to strive for plasma Phe concentrations in the desired target range. This study shows that even if the plasma Phe concentration is above the target range at the first encounter, achieving the target plasma Phe concentration within one week will likely result in improved plasma Phe control for the remainder of the pregnancy. Furthermore, women who were seen by the nutritionist earlier in their pregnancies had a higher likelihood of achieving plasma Phe control by the time of the second encounter.

It is notable that there was no difference in the ability to achieve plasma Phe control by the second encounter with regard to HPA class. Furthermore, the average plasma Phe concentrations for the remainder of the pregnancies after the second encounter were not different between HPA classes. Since women with CP by definition have a higher diagnostic plasma Phe concentration than the women with VP, we anticipated they would also have higher plasma Phe concentrations both at the first encounter and second encounter, as well as for the remainder of the pregnancy. This was not true for this study cohort. Consistent and supportive clinical guidance along with patient commitment, enabled them to achieve and maintain low plasma Phe concentrations, regardless of the severity of their HPA class. This suggests factors beyond endogenous variability in PAH activity contribute to poor dietary control in MHPA pregnancies.

We expected a cohort effect associated with maternal birth year due to the evolutionary changes in treatment guidelines for HPA. We assumed that a potential decrease in the IQ associated with earlier birth decade might affect the adherence to the dietary restrictions during pregnancy. This was not borne out by these data, and may reflect the significant efforts by the patients to adhere to their dietary restrictions during pregnancy, as well as the frequent visits to the metabolic nutritionist.

This study is limited due to its retrospective nature and small sample size. As such, a complete set of intelligence quotient scores for the maternal cohort was not available for each participant. Neuropsychological assessments are being performed on this cohort and their offspring in an ongoing study. The strength in this study lies in the fact that the same team of providers at the same institution was responsible for the clinical management of each subject. In addition, all of the subjects followed the same dietary management guidelines. To our knowledge, this is the first analysis of the influence of a nutritionist's

intervention in achieving and maintaining Phe control during pregnancy in women with HPA.

To avoid poor outcomes of the offspring, metabolic control in MHPA is essential. The Maternal Phenylketonuria Collaborative Study (MPKUCS) showed that when the average plasma Phe concentration is within the target range, there is a lower incidence of microcephaly, growth restriction and congenital heart defects in the offspring [10]. In addition, Waisbren and Azen demonstrated higher intelligence quotient scores and improved behavior in the offspring of MHPA patients who maintained average Phe concentrations within the target range, as well as those who established plasma Phe concentration control early in gestation [17]. Convenient, widely available medical foods and medical care may contribute to removing obstacles in the achievement of metabolic control for women with MHPA.

In conclusion, instituting and maintaining weekly visits to a nutritionist who is experienced in treating MHPA to reinforce and make changes in the diet during pregnancy appears to be a key factor in improving plasma Phe concentrations. From this study, the effect that the intervention of the nutritionist has on plasma Phe concentrations throughout the time of the entire gestation may be predicted by the time of the second encounter. As a result, the first two visits to the nutritionist are extremely important in setting the stage for the remainder of the pregnancy. Every effort to improve dietary compliance in pregnant women with HPA should be made. In our analysis, we have shown that the sooner metabolic control is achieved after counsel from the metabolic nutritionist, the more likely metabolic control will be maintained for the rest of the pregnancy. Consistent metabolic control will lower long-term medical and societal costs resulting from impaired offspring. This manuscript demonstrates the effect that the rapidity of achieving metabolic control has on overall metabolic control throughout pregnancy. An ongoing study to determine the neuropsychological and cognitive outcomes of the offspring from the women in this study is currently underway.

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Highlights

- We studied phenylalanine tolerance in pregnant woman with Hyperphenylalaninemia
- We studied the effect of nutritional intervention in Maternal Hyperphenylalaninemia
- Good metabolic control can be achieved regardless of severity of disease
- Phenylalanine tolerance increases by trimester

Figure 1. Plasma Phe Concentration Throughout Pregnancy, Grouped by Control at Second Encounter

Note horizontal line at 360 $\mu\text{mol/L}$; number above each graph is subject identification.

Figure 2. Phe Trajectories for IT and AT Groups*

* Phe-phenylalanine; IT-within the target range at the Second Encounter; AT-above the target range at the Second Encounter.

Figure 3. Plasma Phe Concentration by Gestational Age for IT and AT Groups*

* Phe-phenylalanine; IT-within the target range at the Second Encounter; AT-above the target range at the Second Encounter.

Table 1**Maternal Demographics by Metabolic Control Status at the Second Encounter**

	In Target (IT), n=25	Above Target (AT), n=10	p-value
Age in years, mean $\pm$ SD	30.6 $\pm$ 4	34.1 $\pm$ 5.3	0.08 ^a
HPA Class, n (%):			
Classical	11 (44)	6 (60)	0.5 ^b
Variant	14 (56)	4 (40)	
Race, n (%):			
Caucasian	22 (88)	10 (100)	0.5 ^b
African-American	3 (12)	0	
Gravidity, median (range)	2 (1–5)	4 (1–11)	0.03 ^c
Parity, median (range)	0 (0–2)	1 (0–4)	0.02 ^c
Number with Nutritional Visit Prior to Conception	6	1	0.6 ^b
Gestational Age at first encounter in weeks, mean $\pm$ SD	4.7 $\pm$ 2.2	6.7 $\pm$ 2.4	0.04 ^a
Gestational Age at delivery in weeks, mean $\pm$ SD	38.2 $\pm$ 1.9	37.4 $\pm$ 3.5	0.6 ^a

^aStudent's t-test^bFisher's Exact Test^cWilcoxon Rank Sum Test

Table 2

Analysis of Phe Tolerance by Trimester

Trimester	Phe Tolerance in Grams/Day, Mean $\pm$ SD *		Ratio of Phe Tolerance VP versus CP (95% Confidence Interval)	Trend in Ratio of Change in Mean Phe Tolerance [†] (95% Confidence Interval)		
	CP [‡]	VP [‡]		Trimesters	CP [‡]	VP [‡]
1 st	7.5 $\pm$ 2.6	12.7 $\pm$ 10.0	1.7 (1.1, 2.6)		---	---
2 nd	10.9 $\pm$ 4	16.8 $\pm$ 9.7	1.5 (1.0, 2.3)	2 nd versus 1 st	1.4 (1.2, 1.8)	1.3 (1.1, 1.5)
3 rd	16.1 $\pm$ 5.2	23.0 $\pm$ 11.0	1.4 (1.1, 1.9)	3 rd versus 2 nd	1.5 (1.2, 1.8)	1.4 (1.0, 1.8)
				3 rd versus 1 st	2.2 (1.6, 2.9)	1.8 (1.3, 2.4)

* Grams/Kilogram/Day

[†]The p-value for the trend in ratio of change is 0.001 for both CP and VP

[‡]CP, Classical Phenylketonuria; VP, Variant Phenylketonuria