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5-year retrospective analysis of patients with phenylketonuria (PKU) and hyperphenylalaninemia treated at two specialized clinics

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ABSTRACT

Background: Phenylketonuria (PKU) is an autosomal recessive disease caused by mutations in the *PAH* gene, resulting in deficiency of phenylalanine hydroxylase (PAH), an enzyme that converts phenylalanine (Phe) to tyrosine (Tyr). The purpose of this study was to capture real-world data associated with managing PKU under current standard of care and to characterize a representative population for a planned gene therapy trial.
Methods: A retrospective chart review was conducted at two U.S. clinics for individuals 10–40 years old diagnosed with PKU-related hyperphenylalaninemia (HPA). Demographics, medical history, treatments and blood Phe data were collected from electronic medical records spanning a five-year period ending in November 2017.
Results: 152 patients were enrolled (65.8% had classical PKU). Although > 95% of patients were prescribed a Phe-restricted diet, blood Phe concentrations remained substantially elevated, particularly in patients diagnosed with classical PKU. As the Phe threshold was lowered (Phe < 600, 360, 120 or 30 μmol/L), the number of patients with consecutive lab values below the threshold decreased, suggesting that many patients' Phe levels are inadequately controlled. 62.5% of patients were reported as having a history of at least one neuropsychiatric comorbidity, and adults were more likely than adolescents (69.5% vs. 54.3%). 92 of 98 PAH genotypes collected were distinct mutations; the 6 null-null genotypes were associated with classical PKU. Overall the demographics and clinical data were consistent across both sites.
Conclusion: Despite dietary restrictions, mean Phe concentrations were > 360 μmol/L (a level considered well-controlled based on current U.S. treatment guidelines) for mild, moderate, and classical PKU patients. There remains an unmet need for therapies to control Phe concentrations.

1. Introduction

Phenylalanine hydroxylase (PAH) deficiency is the primary cause of the inborn error of metabolism, phenylketonuria (PKU). The disease is inherited as an autosomal recessive trait due to mutations on chromosome 12q23.1 [1] in the *PAH* gene that result in the absence or deficiency of PAH activity, an enzyme expressed in hepatocytes that catalyzes the formation of tyrosine (Tyr) from phenylalanine (Phe).

PAH deficiency manifests as a continuum of hyperphenylalaninemia (HPA) phenotypes characterized by elevated blood Phe concentrations. Clinical sub-categories range from mild HPA (Phe levels 120–360 μmol/L) to the most common and severe form, classical PKU, defined as Phe > 1200 μmol/L. Unless treated in childhood, classical PKU leads to progressive impairment of cerebral function and is further associated with behavioral abnormality, seizures, mental retardation, eczema, asthma, headaches, and various psychiatric disorders [2].

Abbreviations: ACMG, American College of Medical Genetics and Genomics; ANCOVA, analysis of covariance; ANOVA, analysis of variance; BCH, Boston Children's Hospital; CHP, University of Pittsburgh Medical Center Children's Hospital of Pittsburgh; HPA, hyperphenylalaninemia; GEE, generalized estimating equation; GMP, glycomacropeptide; IRB, Institutional Review Board; LNAA, large neutral amino acids; PAH, phenylalanine hydroxylase; Phe, phenylalanine; PKU, phenylketonuria; Tyr, tyrosine

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With the advent of newborn screening in 1963, managing the disease with a low Phe diet in infants before clinical symptoms appear became the standard of care. However, the diet is highly restrictive of natural protein sources and requires supplementation with medical foods to ensure adequate intake of other amino acids. While dietary management has its most dramatic effects during infancy and childhood, it must continue in adolescents and adults for ongoing management of the blood Phe concentration. Current treatment guidelines have reaffirmed the need to maintain lifelong dietary restrictions. Adults who fail to adhere to a Phe-restricted diet have been demonstrated to experience a reduction in IQ [3]. In reality, the vast majority of adults with classical PKU have Phe levels far in excess of the maximum of 360 μmol/L, recommended by the American College of Medical Genetics and Genomics (ACMG) [1], or even the more lenient < 600 umol/L recommendation for those over the age of 12 years recommended by the European guidelines [4]. As a result, many suffer from significant central nervous symptoms including suboptimal attention, processing speed, and reaction time [5]. Sapropterin dihydrochloride (sapropterin) (Kuvan®, BioMarin Pharmaceutical) reduces Phe in only a very few patients with classical PKU; consequently, essentially all must remain on a reduced Phe diet to maintain recommended treatment levels [6–8]. Pegvaliase, an enzyme substitution therapy consisting of phenylalanine ammonia lyase (PAL) conjugated to polyethylene glycol (PEG) has recently been approved for treatment of adults with PKU but requires daily injections and has the risk of allergic and inflammatory reactions. Two alternative dietary interventions are available to PKU patients: glycomacropeptide (GMP) and large neutral amino acid (LNAA) supplements. GMP is a naturally low Phe protein source that is a byproduct of cheese making and is used in medical foods in place of other natural proteins or amino acid mixtures [9]. LNAA mixtures that do not contain Phe may decrease transport of Phe into the brain and may be given to patients who cannot tolerate the diet [10]. However, efficacy of LNAA is inconsistent and dietary restriction of Phe intake is still recommended [6].

Even with prescribed diets and supplements, it has historically been difficult for patients to maintain Phe concentrations within an ideal range. In a recent survey, less than half of respondents in the US had levels < 360 μM [11]. The survey also identified an increased difficulty managing blood Phe for adults compared with adolescents. Additionally, 91.4% of respondents reported that development of new PKU treatments is somewhat important, important, or very important, clearly demonstrating that there is an unmet need for therapies for this disorder.

We have performed a retrospective chart review of patients with a diagnosis of PKU-related HPA using electronic medical records spanning a 5-year period ending in November 2017 to capture real-world data associated with managing PKU under current standard of care. The primary objectives of the study were to characterize blood Phe control in individuals with HPA over the 5-year period; to understand the ability of patients to achieve target blood Phe concentrations relative to current treatment guidelines and therapeutic goals; and to characterize a representative population for a planned gene therapy trial.

2. Patients and methods

2.1. Study population

152 patients with a diagnosis of HPA were included in a retrospective medical chart review. Patients were treated at either Boston Children's Hospital (BCH) or the University of Pittsburgh Medical Center Children's Hospital of Pittsburgh (CHP) and were 10–40 years of age at the time of the review. The study aimed to capture real-world data associated with the PKU population being managed under current standard of care and characterize blood Phe control. Patients who participated in blinded investigative studies or who had fewer than 2 visits at the sites over the study period were excluded. The study was reviewed and approved by the Institutional Review Boards (IRB) at BCH and the CHP. A Waiver of Informed Consent and HIPAA was granted by each IRB due to the minimal risk and retrospective nature of the research.

2.2. Data collection

Data were retrospectively collected from electronic medical records; baseline was defined as the time when patient record collection began. The study reviewed records from baseline to 5 years prior to baseline +/− 3 months. Data collected included birthdate and date of baseline assessment, sex, height, weight, HPA diagnosis date and subtype, PAH genotype (as available), neuropsychiatric diagnoses, medical history, sapropterin dosing and adherence, concomitant medications, known investigational therapies used to treat HPA, dietary prescription (prescriptions for daily energy, protein, Phe, protein equivalent, medical food intake as available), and Phe and Tyr concentrations measured across the study period.

2.3. Data analysis

Due to the nature of the work as a retrospective chart review, there were no universal fixed timepoints for collection of individuals' Phe and Tyr lab values. In order to summarize these data over time, values were aggregated in 90 day and 1-year periods across the study. Phe trends were analyzed across the study and were also grouped by sex, age, HPA diagnosis, and sapropterin use for further analyses. Age was grouped as 10–18 and > 18–40. HPA diagnoses (at the time of diagnosis) were classical PKU (Phe > 1200 μmol/L), Moderate PKU (900–1200 μmol/L), Mild PKU (600–900 μmol/L), and mild HPA-gray zone (360–600 μmol/L). Trends in variation by sex were reassessed while excluding females who were pregnant during the study. Phe levels above 360 μmol/L were considered to be above the threshold for controlled blood Phe per the ACMG guidelines.

Generalized estimating equation (GEE) models were used to assess the data to identify a relationship between control of blood Phe and each of several potential predictor variables: PAH genotype, HPA diagnosis, age, sex, diet, consumption of medical foods or protein equivalents, and use of sapropterin. Univariable (GEE) linear regression was used in the cases of age, diet, and diagnosis and univariable GEE analysis of variance (ANOVA) was used for sex, age group, classical PKU, and sapropterin use. Multivariate GEE analysis of covariance (ANCOVA) was used to determine correlations between Phe control across multiple covariates. *P*-values were considered significant if < 0.1 for univariate analysis and < 0.05 for multivariate analysis; there was no imputation of missing data.

3. Results

3.1. Baseline demographics and disease characterization

The systematic chart review of patients' records pulled Phe measurements from the 152 patients with a mean study duration of 3.9 years as shown in Table 1. A total of 4519 Phe values were collected over the course of the study and, on average, patients contributed approximately 30 Phe values to the dataset. Demographics and diagnoses, and prescribed treatment of study patients are shown in Tables 2 and 3, respectively. Patients included 77 females and 75 males and the majority (91.4%) were Caucasian. Classical PKU was the most common HPA diagnosis and mild PKU was the second most common (65.8% and 17.1% of overall population respectively). Age was balanced between the 10–18 and > 18–40 populations at 46.1% and 53.9% respectively. Baseline patient demographics, treatment, and other observations were consistent across study sites. There were no patients taking an investigational therapy for HPA.

The presence of baseline dietary restrictions was high across study

Table 1
Patient accountability.

	Boston	Pittsburgh	All
Patients enrolled	$N = 65$	$N = 87$	$N = 152$
Total number of Phe values collected	1637	2882	4519
Number of Phe values per patient			
Mean +/− SD	25.2 ± 27.6	33.1 ± 27.4	29.7 ± 27.7
Median (min, max)	13.0 (2.0, 114.0)	26.0 (2.0, 143.0)	20.5 (2.0, 143.0)
Study duration per patient (years)			
Mean +/− SD	3.5 ± 1.5	4.2 ± 1.0	3.9 ± 1.3
Median (min, max)	4.1 (0.1, 5.2)	4.5 (0.9, 5.0)	4.4 (0.1, 5.2)

Table 2
Baseline demographics.

Characteristic	10–18	> 18-40	All
	$N=70$[a]	$N=82$[a]	$N=152$[a]
Age at baseline, years, mean (SD)	14.2 (2.3)	25.3 (4.6)	20.2 (6.7)
Sex, female, n (%)	36 (51.4%)	41 (50.0%)	77 (50.7%)
Race, n (%)			
White	64 (91.4%)	75 (91.5%)	139 (91.4%)
Black/African American	1 (1.4%)	1 (1.2%)	2 (1.3%)
Other	5 (7.1%)	6 (7.3%)	11 (7.2%)
HPA Diagnosis, n (%)			
Classical PKU	45 (64.3%)	55 (67.1%)	100 (65.8%)
Moderate PKU	6 (8.6%)	10 (12.2%)	16 (10.5%)
Mild PKU	12 (17.1%)	14 (17.1%)	26 (17.1%)
Mild HPA-gray zone	7 (10.0%)	3 (3.7%)	10 (6.6%)
Height at −5 years, cm, median (range)	136.7 (108.1 to 175.5)	168.3 (141.0 to 189.0)	157.0 (108.1 to 89.0)
Height at baseline, cm, median (range)	157.7 (133.4 to 193.0)	168.9 (145.0 to 190.0)	163.2 (133.4 to 193.0)
Weight at −5 years, kg, median (range)	32.7 (18.9 to 93.2)	65.5 (34.5 to 128.4)	53.0 (18.9 to 128.4)
Weight at baseline, kg, median (range)	52.6 (29.6 to 127.0)	72.2 (43.0 to 147.8)	64.4 (30.3 to 147.8)
BMI at −5 years, median (range)	17.5 (14.0 to 34.0)	24.0 (16.0 to 41.0)	21.0 (14.0 to 41.0)
BMI at baseline, median (range)	21.0 (15.0 to 37.0)	26.0 (16.0 to 51.0)	23.0 (16.0 to 51.0)

[a] Population size unless specified otherwise in characteristic header.

subgroups with the majority reporting restrictions regardless of HPA subtype, age, sex, and study site; 95.4% of all study patients were on a restricted diet. Dietary Phe intake and medical food consumption prescribed as protein equivalents was similar across the sites for the overall study cohort. Patient adherence to diet on the basis of Phe, protein, and medical food intake was recorded as available, but the availability of this data was limited (only 37.2%, 31.7%, and 42.1% of patients on dietary restrictions reported adherence data respectively). Of those that did report their adherence, a higher proportion of female subjects reported that they were adherent to daily protein intake (45.0% vs 34.6%), daily Phe intake (63.6% vs 31.3%), and medical food consumption (55.6% vs 50.0%) when compared to male patients.

Half of all study patients (76/152) reported taking sapropterin at some point during the 5-year study period. This was consistent across both sites (53.8% in Boston vs. 47.1% in Pittsburgh) and age groups (54.3% 10–18 years old vs 46.3% > 18–40 years old) but varied by diagnosis (45.0% classical PKU vs 59.6% non-classical PKU). A minority of the patients reported on their medication adherence ($n = 69$) and duration of sapropterin use ($n = 37$) making a determination of whether the medication use was sustained difficult.

Neuropsychiatric history, as shown in Table 4, was captured at baseline. A history of having at least one neuropsychiatric condition was reported in 62.5% of patients and 44.1% of patients had more than one neuropsychiatric diagnosis. The most common was anxiety (38.2%) and the second most common was inattention (23.0%). Adults were more likely than adolescents to have a neuropsychiatric comorbidity (69.5% vs. 54.3%) and even more likely than adolescents to have multiple of these comorbidities (54.9% vs. 31.4%). No differences in baseline characteristics or medical history were observed across sites, suggesting a similar patient population.

Table 4
Neuropsychiatric history.

	10–18	> 18–40	All
	$N = 70$	$N = 82$	$N = 152$
Any history n (%)	38 (54.3%)	57 (69.5%)	95 (62.5%)
> 1 Diagnosis[a] n (%)	22 (31.4%)	45 (54.9%)	67 (44.1%)
Diagnosis[a] n (%)			
Anxiety	21 (30.0%)	37 (45.1%)	58 (38.2%)
Behavioral disturbances	12 (17.1%)	12 (14.6%)	24 (15.8%)
Depression	5 (7.1%)	27 (32.9%)	32 (21.1%)
Hyperactivity	13 (18.6%)	9 (11.0%)	22 (14.5%)
Inattention	12 (17.1%)	23 (28.0%)	35 (23.0%)
Irritability	7 (10.0%)	10 (12.2%)	17 (11.2%)
Seizure	2 (2.9%)	2 (2.44%)	4 (2.6%)
Tremors	4 (5.7%)	3 (3.7%)	7 (4.6%)
Psychosis	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gait disturbance	1 (1.4%)	0 (0.0%)	1 (0.7%)
Executive function deficits	3 (4.3%)	15 (18.3%)	18 (11.8%)

[a] Patients were counted if they had any history with the condition.

Table 3
Baseline dietary characteristics.

Characteristic	10–18	> 18-40	All
	$N=70$[a]	$N=82$[a]	$N=152$[a]
Sapropterin use during study period, n (%)	38 (54.3%)	38 (46.3%)	76 (50.0%)
Patient reported adherence to sapropterin, % (n/N)	77.1% (27/35)	64.7% (22/34)	71.0% (49/69)
Dose, mg/day, mean (SD) [N]	931.9 (361.5) [38]	1401.8 (409.2) [38]	1166.8 (450.6) [76]
Time on medication, years, mean (SD) [N]	4.8 (2.5) [19]	2.6 (1.5) [18]	3.7 (2.3) [37]
On dietary restrictions, n (%)	66 (94.3%)	79 (96.3%)	145 (95.4%)
Dietary Phe intake, mg/kg/day, mean (SD) [N]	14.2 (18.5) [60]	8.7 (8.1) [64]	11.4 [124]
Dietary Phe intake, mg/day, mean (SD) [N]	675.0 (748.9) [60]	626.6 (611.8) [64]	650.0 [124]
Patient reported adherence to dietary Phe restriction, % (n/N)	40.0% (8/20)	47.1% (16/34)	44.4% (24/54)
Total protein intake, g/kg/day, mean (SD) [N]	1.1 (0.3) [53]	1.0 (0.3) [73]	1.0 (0.3) [126]
Total protein intake, g/day, mean (SD) [N]	62.3 (18.2) [53]	71.0 (18.1) [74]	67.4 (18.5) [127]
Patient reported adherence to dietary protein restriction, % (n/N)	23.1% (3/13)	45.5% (15/33)	39.1% (18/46)
Protein equivalents from medical food consumption, g/kg/day, mean (SD) [N]	1.0 (0.8) [60]	0.8 (0.3) [73]	0.9 (0.6) [133]
Protein equivalents from medical food consumption, g/day, mean (SD) [N]	57.2 (57.1) [60]	58.3 (20.7) [74]	57.8 (41.0) [134]
Patient reported adherence to dietary medical food consumption, % (n/N)	47.8% (11/23)	55.3% (21/38)	52.5% (32/61)

[a] Population size unless specified otherwise in characteristic header.

Table 5
PAH genotyping by HPA diagnosis.

	Classical PKU	Non-classical PKU	All
PAH genotype collected [% (n/N subj)]	66.0% (66/100)	61.5% (32/52)	64.5% (98/152)
Missense-missense	56.1% (37/66)	62.5% (20/32)	58.2% (57/98)
Missense-null	34.8% (23/66)	37.5% (12/32)	35.7% (35/98)
Null-null	9.1% (6/66)	0.0% (0/32)	6.1% (6/98)
Chi-square[a]	$p = .21$		
Unknown or not collected [% (n/N subj)]	34.0% (34/100)	38.5% (20/52)	35.5% (54/152)

[a] p-value from Chi-square test of proportions to test association between available PAH genotypes and PKU diagnosis (classical vs. non-classical).

PAH genotype (Table 5.) was known for 64.5% of the study population ($n = 98$). Of those collected, there were 90 distinct mutations; 57 (58.2%) were missense-missense mutations, 35 (35.7%) were missense-null mutations, and 6 (6.1%) were null-null mutations. While all 6 null-null mutations were found in the classical PKU population, there was no correlation between the missense-missense and missense-null mutations and PKU diagnosis ($p = .21$).

3.2. Phe concentrations over time

The mean Phe concentration overall was 583.6 μmol/L with a higher value in adults than adolescents (694.7 vs. 456.8 μmol/L respectively), as seen in Table 6. For both age groups, the mean Phe concentration of the classical PKU population was above the threshold of 360 μmol/L for optimal control although a few patients did have mean values within the well-controlled range (26.7% in adolescents and 7.3% in adults). Phe was better controlled in the non-classical PKU population with 72.0% of adolescents and 51.9% of adults having a mean Phe concentration below 360 μmol/L. Notably, adult patients had a higher Phe concentration than adolescents in all categories.

Phe values from across the study were pooled into 90-day intervals with the error bars indicating a spread of one standard deviation (Fig. 1). The whole population Phe concentration was generally stable over time with mean values of 590 μmol/L from year −5 to −4 and 611 μmol/L from year −1 to baseline. Since the mean was found to be consistently above the threshold for well controlled Phe of 360 μmol/L, additional trends were investigated by filtering into subpopulations.

Fig. 1 displays the mean Phe (μmol/L) and standard error values over time across time windows of 90-day intervals. A dotted reference line at 360 μmol/L is included for comparison.

Overall Phe values were found to be consistently higher among individuals with a diagnosis of classical PKU when compared to those with non-classical PKU as seen in Fig. 2. This elevation was found in both male and female patients as seen in Fig. 3.

When assessing the Phe concentration over time, it was found that there was a separation between age groups, as shown in Fig. 4. This dichotomy became more apparent after restricting the sample to exclude those with non-classical PKU and females who were pregnant. Both age groups were consistently above the threshold for a well-controlled Phe concentration, but the > 18–40 adult group consistently had a higher mean Phe concentration level than the 10–18 adolescent population.

Fig. 2 displays the mean Phe (μmol/L) and standard error values over time across time windows of 90-day intervals. The data is displayed for classical PKU and non-classical PKU groups. A dotted reference line at 360 μmol/L is included for comparison.

Fig. 3 displays the mean Phe (μmol/L) and standard error values over time across time windows of 90-day intervals. The data is displayed by sex in the Classical PKU group, excluding females that were pregnant during the data collection period. A dotted reference line at 360 μmol/L is included for comparison.

Fig. 4 displays the mean Phe (μmol/L) and standard error values over time across time windows of 90-day intervals. The data is displayed by age grouping (10–18 vs > 18–40) in the Classical PKU group, excluding females that were pregnant during the data collection period. A dotted reference line at 360 μmol/L is included for comparison.

Phe trends were also assessed for the population of patients taking sapropterin at any time during the 5-year period (the data do not account for the duration or timing relative to the study period). While Phe values were lower for patients taking sapropterin, it was found that even for these patients, Phe concentrations were above the threshold.

3.3. Phe/Tyr ratio over time

The ratio of Phe to Tyr was also examined. In healthy individuals, the ratio is approximately 1:1. For PAH deficiency, it ranges from 2.5:1 to well above 10:1 in classical PKU cases [12]. Using a cutoff of 2.5, patients within both the classical and non-classical PKU groups were found to have an elevated ratio over the course of the study with the classical PKU group being the higher of the two groups as shown in Fig. 5.

Within the classical PKU group, those in the > 18–40-year-old adult population had a higher ratio than the 10–18 year old adolescent population, as shown in Fig. 6.

Table 6
Mean Phe values and patients above recommended Phe levels.

	10–18	> 18–40	All
	70 Patients	82 Patients	152 Patients
	2665	1797	4462
	Observations	Observations	Observations
Overall population, Phe across study, μmol/L, mean (SD)	456.8 (27.0)	694.7 (36.7)	583.6 (25.2)
Patients with mean Phe across study > 360 μmol/L, % (n)	57.1% (40)	78.0% (64)	68.4% (104)
- Classical PKU, Phe across study, μmol/L, mean (SD)	528.1 (34.8)	798.8 (41.2)	676.1 (30.6)
Patients with mean Phe across study > 360 μmol/L, % (n/N)	73.3% (33/45)	92.7% (51/55)	84.0% (84/100)
- Non-classical PKU, Phe across study, μmol/L, mean (SD)	326.3 (27.0)	465.1 (50.3)	391.6 (28.8)
Patients with mean Phe across study > 360 μmol/L, % (n/N)	28.0% (7/25)	48.1% (13/27)	38.5% (20/52)

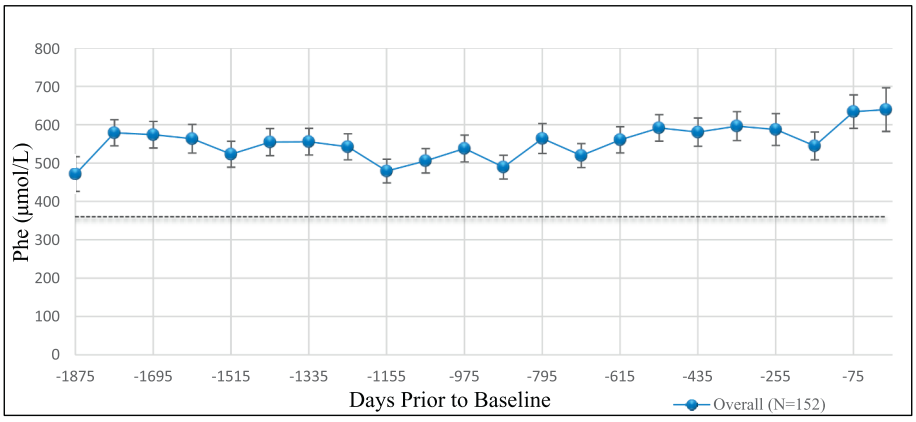

Fig. 1. Mean Phe over time, full population.

Fig. 5 displays the mean Phe/Tyr ratio and standard error values over time across time windows of 90-day intervals. The data is displayed to compare classical PKU with non-classical PKU. A dotted reference line at 2.5 is included for comparison.

Fig. 6 displays the mean Phe/Tyr ratio and standard error values for classical PKU over time across time windows of 90-day intervals displayed by age grouping (10–18 vs > 18–40) (excluding females that were pregnant during the data collection period). A dotted reference line at 2.5 is included for comparison.

3.4. Univariate and covariate analysis

As shown by the univariate analysis (Table 7), the Phe concentration was found to increase with age, and higher Phe concentration was associated with male sex and with classical PKU. Conversely, lower Phe concentration was associated with sapropterin use, protein restrictions, and consumption of medical food.

In the multivariate analysis shown in Table 8, age, male sex, and classical PKU were positively associated with Phe concentration and medical food consumption was negatively associated with Phe concentration. Sapropterin use was not a significant predictor of the Phe concentration after accounting for these other covariates and was removed from the model.

4. Discussion

This 5-year retrospective chart review was conducted at two specialized clinics, Boston Children's Hospital and the UPMC Children's Hospital of Pittsburgh in anticipation of a gene therapy trial. A total of 152 patients (10 to 40 years old) with a diagnosis of HPA were included in the study. The primary objective was to describe blood Phe levels and the degree of metabolic control in these patients under current standard of care over a 5-year period and to characterize a representative population for a planned gene therapy trial.

Overall, Phe concentrations in this population were consistently elevated over the 5-year period, with a mean value of 583.6 ± 25.2 µmol/L. Most of the patients were diagnosed with classical PKU (65.8%). Patients with classical PKU had distinctly higher mean Phe concentrations (676.1 ± 30.6 µmol/L) relative to non-classical. Only patients with a diagnosis of mild HPA had a mean Phe level in the range considered well-controlled (≤360 µmol/L). A dichotomy was found between adolescent and adult individuals, with adults having higher Phe concentrations which was in agreement with prior studies [11]. These data demonstrated a relationship between increased age and decreasing control of Phe concentrations. Even though adolescents tended to have more controlled Phe, the majority of these patients were consistently above the threshold for well controlled Phe of 360 µmol/L, which did not vary significantly across sites or over

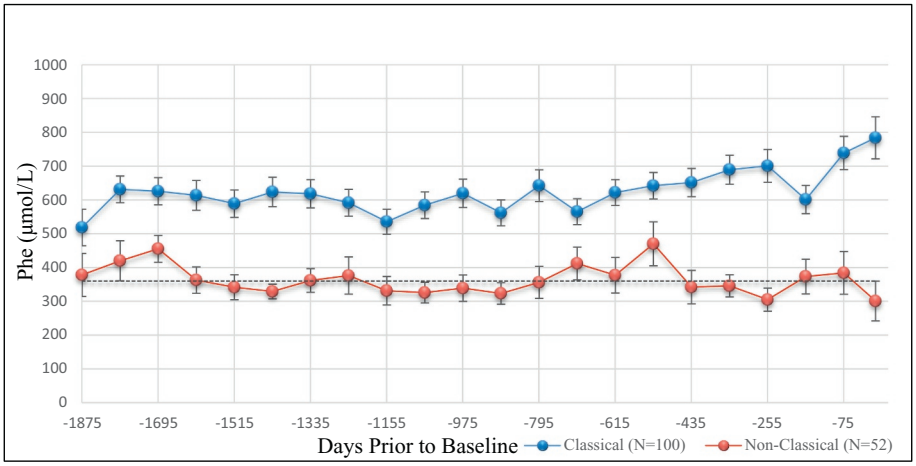

Fig. 2. Mean Phe over time by PKU diagnosis (classical vs non-classical).

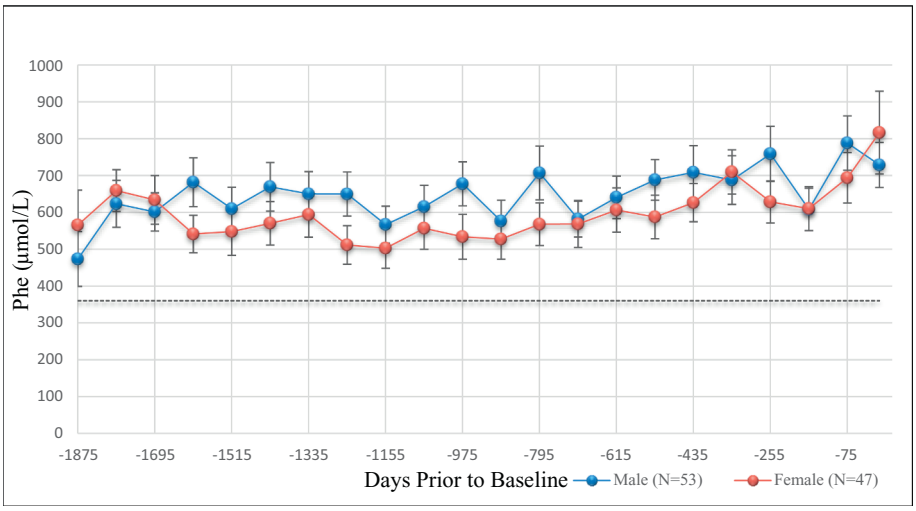

Fig. 3. Mean Phe over time by sex, classical PKU patients only, pregnant females censored.

time. There were no clinically relevant differences in Phe concentrations over time in males versus females (although females trended lower). The multivariate analysis revealed that increasing age, male sex, and a diagnosis of classical PKU all resulted in a statistically significant increase on Phe values. Increasing medical food consumption resulted in a statistically significant decrease in Phe values.

Despite nearly every patient (> 94%) having been prescribed dietary measures to control blood Phe, the concentration remained at an elevated level compared to treatment guidelines, particularly in patients with classic PKU. This is true even for patients taking sapropterin cofactor therapy during the 5-year period, including the majority (71%) who reported adherence to their prescription, consistent with earlier studies [13]. Few patients reported on their adherence to their prescribed diets, but of those who did, less than half claimed to have followed their dietary restrictions. This supports the notion that maintaining a strict lifelong diet is challenging for many and that a prescribed diet alone is often insufficient for achieving Phe goals. New treatments should aim to circumvent the need for dietary controls to more reliably keep the Phe within well-controlled levels.

In addition to elevated Phe concentrations, 62.5% of patients reported as having a history of at least one neuropsychiatric condition and 44.1% of patients were recorded as having two or more neuropsychiatric conditions. Adults were more likely to have a neuropsychiatric comorbidity (69.5% vs. 54.3%) and were much more likely to have multiples of these comorbidities (54.9% vas. 31.4%). The inability to achieve normal levels of Phe has been associated with neurological problems (1). We did not assess all of the potential comorbidities that have been reported in PKU, including comorbidities in somatic organs as reported from the United States [14] and Germany [15], but these can add to the negative impact of PKU in the patient.

Which of the 152 patients we retrospectively assessed may be candidates for gene therapy? We believe that all patients with PKU whose blood Phe concentration usually or continually exceeds 360 µmol/L should be considered for this therapy if it is shown to be safe and successful. However, we recognize that other factors are likely to also influence the decision to undergo gene therapy. A major general factor would be the perceived burden of PKU. Patients for whom the diet is an onerous factor in their lives would likely welcome a therapy that promises to allow a completely normal diet. Likewise, patients who believe that PKU has adversely affected their functioning in school and the workplace or in their social relationships might welcome a therapy that could reverse or reduce these difficulties. There also are likely to be patients who are concerned that PKU might predispose to later neurological disorders such as Parkinson's or Alzheimer's disease [16] and

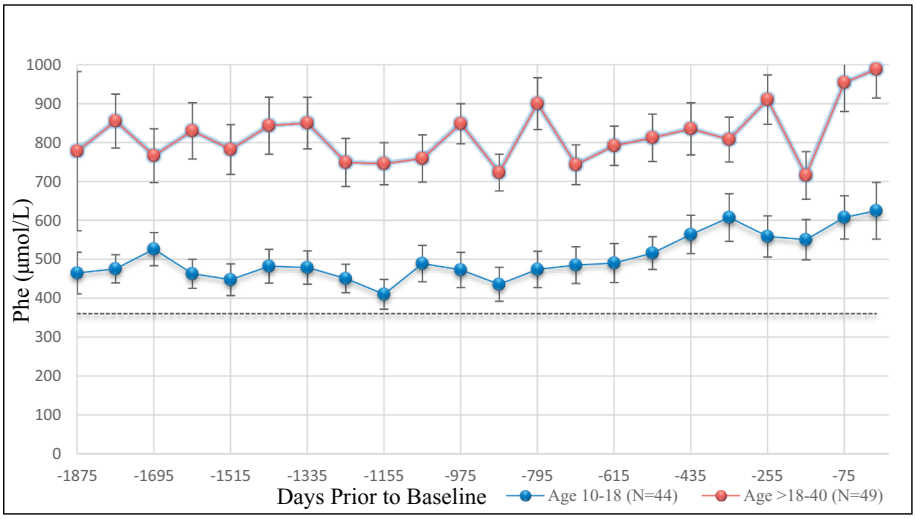

Fig. 4. Mean Phe over time by age group, classical PKU patients only, pregnant females excluded.

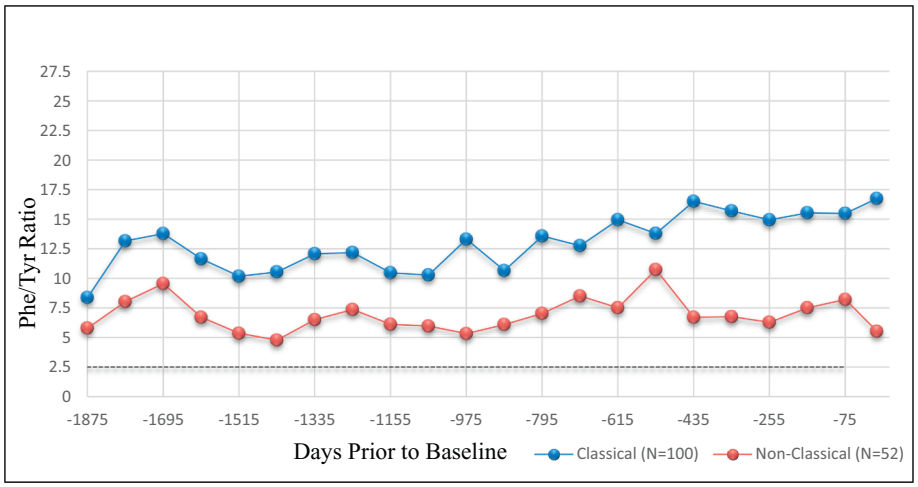

Fig. 5. Mean Phe/Tyr ratio over time by PKU diagnosis (classical vs non-classical).

believe that gene therapy might reverse this predisposition. Thus, while many factors are likely to influence the decision to undergo gene therapy, the entrance into the decision process will be control of blood Phe. However, the rare patient who may have escaped the cognitive or other neurological features of PKU despite a consistently elevated Phe concentration and little or no therapy [17] would likely not consider gene therapy or be considered by the scientific community as a candidate for this therapy.

The data were analyzed further to assess Phe control in the patient population. The results showed that only 50.7% (77/152) of patients had 2 consecutive observations < 360 μmol/L and only 13.2% (20/152) of patients had 2 consecutive observations < 120 μmol/L. For individuals with classical PKU, the percentages were 34.4% (32/93) and 14.0% (13/93), respectively. In addition, most patients had mean Phe levels > 360 μmol/L (68.4%), especially adult patients with classical PKU (92.7%). These findings suggest that the majority of patients are unable to achieve sustained Phe control or maintain Phe levels in the recommended range.

In 2018, Pegvaliase-pqpz (pegvaliase) (Palynziq®, BioMarin Pharmaceuticals), an enzyme substitution therapy, was approved by the FDA for reduction of blood Phe concentration in adults with Phe concentrations above 600 μmol/L. This enzyme metabolizes Phe into trans-cinnamic acid and ammonia [18]. Approval of this therapy occurred outside the time window of the retrospective chart review, so none of the individuals in this study used this medication during the study period.

PAH deficiency is known to be caused by genetic mutations in the *PAH* gene. There are over 2500 known genotypes that result in HPA [19]. When grouping the genotypes reported in this study by the type of mutation (e.g. missense-null), it was found that all 6 null-null mutations were associated with a diagnosis of classical PKU. Trends were not apparent in the other groupings. The most common genotype in this study (R408W/R408W) was observed in just four individuals (all with a diagnosis of classical PKU). We did observe that the homozygote R408W mutation and compound heterozygote mutations that included other known loss-of-function mutations were associated with classical

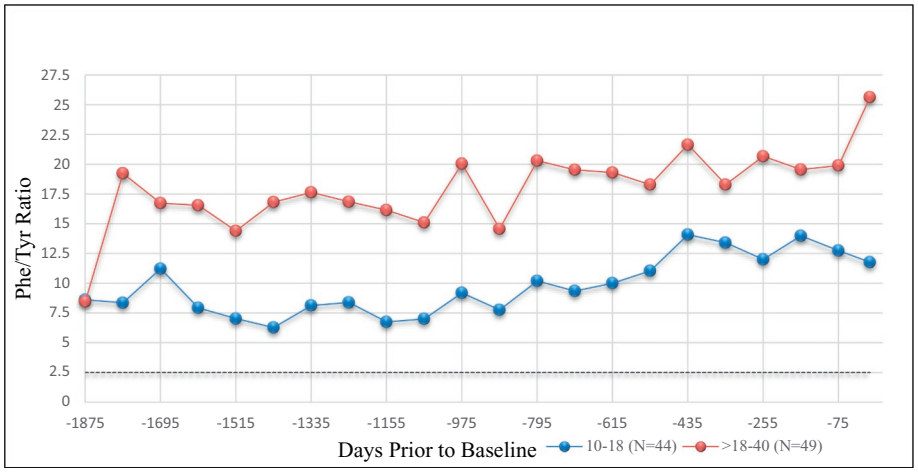

Fig. 6. Mean Phe/Tyr ratio over time by age group, classical PKU patients only, pregnant females censored.

Table 7
Univariate analysis of age, sex, energy intake, protein restriction, dietary phenylalanine intake, consumption of medical foods, sapropterin use, and diagnosis.

	Regression estimate for Phe (95% CI)	P-value
Age (years)	17.2 (10.2, 4.2)	< 0.0001*
Age group 18–30 (10–18 = reference)	237.8 (143.9, 331.8)	< 0.0001*
Age group 30–40 (10–18 = reference)	229.5 (32.6, 426.3)	0.0223*
Sex (female = reference)	185.6 (90.4, 280.8)	0.0001*
Sex (pregnancy censored, female = reference)	176.1 (76.8, 275.4)	0.0005*
Energy intake (kcal/kg/day)	−0.99 (−2.10, 0.12)	0.0812 *
Total protein intake (g/kg/day)	−77.7 (−169.9, 14.5)	0.0985 *
Dietary phenylalanine intake (mg/kg/day)	−0.32 (−1.58, 0.93)	0.6153
Medical food consumption-protein equivalent (g/kg/day)	−52.2 (−96.4, −8.0)	0.0205 *
Sapropterin use (not used = reference)	−97.6 (−194.9, −0.4)	0.0491*
Diagnosis	140.4 (106.0, 174.9)	< 0.0001*
Classical PKU (non-classical = reference)	271.1 (184.3, 357.8)	< 0.0001*

* Indicates significance at $p < .10$.

Table 8
Multivariate analysis of age, sex, diagnosis, and consumption of medical foods.

	Adjusted regression estimate for Phe (95% CI)	P-value
Age (years)	17.0 (10.5, 23.4)	< 0.0001*
Sex (female = reference)	169.0 (83.0, 254.9)	0.0001*
Classical PKU (non-classical = reference)	209.0 (123.3, 294.7)	< 0.0001*
Medical food consumption-protein equivalent (g/kg/day)	−52.0 (−95.8, −8.3)	0.02*

* Indicates significance at $p < .05$

PKU in this population, which was in agreement with the findings of Himmelreich et al. [19]. However, due to the limited data set and variety of distinct mutations (92 out of 98), many of which have not been characterized for effect on function, correlations between genotype and phenotype in this study were not statistically significant. Despite the many genotypes, PAH deficiency is inherited as an autosomal recessive, monogenic condition. This makes it potentially suitable for a gene therapy approach to provide functional copies of the PAH gene to the liver, but this would require further study.

5. Conclusions

Collectively, these real-world data show that Phe concentrations remain elevated above the target therapeutic threshold (360 μmol/L) with current standard of care. Thus, there is significant unmet medical need for therapies to control blood Phe in patients with PKU resulting from PAH deficiency. Prescription of diet, sapropterin, medical foods, and protein equivalents may reduce worsening of PKU, but do not consistently reduce Phe concentrations to normal levels or to the level recommended in the current treatment guidelines. New therapies are needed that eschew reliance on lifelong dietary controls as patients have difficulty maintaining the highly restrictive diet and diet alone is often insufficient for achieving Phe goals.

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