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Maximal dietary responsiveness after tetrahydrobiopterin (BH4) in 19 phenylalanine hydroxylase deficiency patients: What super-responders can expect

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ABSTRACT

Background: Inherited phenylalanine hydroxylase deficiency, also known as phenylketonuria (PKU), causes poor growth and neurologic deficits in the untreated state. After ascertainment through newborn screen and dietary phenylalanine (Phe) restriction to achieve plasma Phe in the range of 120–360 μmol/L, these disease manifestations can be prevented. Poor compliance with protein restricted diets supported by medical food is typical in later years, beginning in the late toddler and teenage years. Pharmacologic doses of oral tetrahydrobiopterin (BH4; sapropterin dihydrochloride) is effective in reducing plasma Phe in about 40–50% of PKU patients but effectiveness is highly variable.

Objective: To assess the maximal responsiveness to 20 mg/kg/day oral BH4 as it affects plasma Phe and dietary Phe allowance in PKU patients.

Materials and methods: This was a single-center, retrospective observational study, combining case reports of individual patients. We reported an outcome of 85 patients with PKU who were trialed on BH4. Phe levels and dietary records of 19 BH4 "super-responders" were analyzed.

Results: Overall, 63.5% of the patients (54/85) were considered BH4 responders. However, we quantitated the dietary liberalization of 19 of our responsive patients (35%), those with at least a 2-fold increase in dietary Phe and maintenance of plasma Phe in treatment range. In these "super-responders", the mean plasma Phe at baseline was 371 ± 237 μmol/L and decreased to 284 ± 273 μmol/L after 1 year on BH4. Mean dietary Phe tolerance increased significantly from 595 ± 256 to 2260 ± 1414 mg/day ($p \leq 0.0001$), while maintaining mean plasma Phe levels within treatment range. Four patients no longer required dietary Phe restriction and could discontinue medical food. The majority of patients had at least one BH4-responsive genotype.

Conclusion: This cohort demonstrates the maximally achievable dietary liberalization which some PKU patients may expect with BH4 therapy. Health benefits are considered to accrue in patients with increased intact protein.

1. Introduction

Phenylketonuria (PKU; OMIM#261600) is a panethnic, autosomal recessive inborn error of metabolism affecting 1:10,000–1:15,000 live births in the United States [1,2]. PKU is caused by pathogenic mutations in the phenylalanine 4-hydroxylase (*PAH*) gene which codes for the phenylalanine hydroxylase (PAH) enzyme. PAH is a tetrahydrobiopterin (BH4)-dependent enzyme that catalyzes the hydroxylation of Phe to tyrosine in the liver [3]. Deficiency of PAH enzyme activity causes increased plasma concentration of Phe, which is neurotoxic, leading to intellectual disability, epilepsy, and white matter changes [4,5]. The patients with the most severe PAH deficiency or classical PKU have untreated plasma Phe above 1200 μmol/L. Patients considered to have nonclassical PKU, with milder phenotypes including mild PKU and mild hyperphenylalaninemia (HPA), have untreated (no dietary restriction) plasma Phe of 600–1200 μmol/L and <600 μmol/L, respectively [6].

Abbreviations: PAH, phenylalanine hydroxylase;; PKU, phenylketonuria;; Phe, phenylalanine;; BH4, tetrahydrobiopterin.

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Early diagnosis and dietary protein restriction are essential to reduce plasma Phe and to prevent irreversible brain damage. Newborn screening allows identification of infants with PKU [7] and initiation of the standard of care within the first few weeks of life. PKU treatment includes a diet restricted in Phe (obtained from intact protein food sources) that is supplemented with a Phe-free medical food with the goal of maintaining plasma Phe in the range of 120–360 μmol/L [6].

BH4 supplementation enhances residual PAH enzyme activity and increases metabolism of plasma Phe to tyrosine in some patients with PKU [3]. In 1999, oral BH4 therapy was demonstrated to reduce plasma Phe in PKU patients [8]. Reduction in plasma Phe after BH4 therapy may be seen within a day of treatment [9], however, some patients require longer than 48–96 h to respond [10–14]. In 2007, sapropterin dihydrochloride (Kuvan®) a synthetic form of BH4 available in oral formulation to treat PKU, was approved by the US Food and Drug Administration. The majority of patients with non-classical PKU respond to oral administration of BH4, while only 10% of classical PKU patients respond to BH4 [15]. Genotype is sometimes useful in predicting BH4 responsiveness [15–17]. Arg408Trp, Arg261Gln, Leu48Ser, and Arg158Gln were frequently reported as BH4 responsive variants [15,18,19]. BH4 has been widely used as a concomitant therapy to a Phe restricted diet to improve metabolic control and increase natural protein tolerance [20–22]. BH4 responders are often able to at least double their dietary Phe intake and maintain plasma Phe levels in treatment range within the first few months after initiating BH4 therapy [20,22,23]. In addition, a subset of patients no longer require medical food while maintaining plasma Phe levels in the target range [20,24–26]. A more normal diet has been proposed as benefiting patients with improved nutritional status as well as quality of life [27].

In this study, we describe the maximal dietary benefit in a cohort of 19 patients who responded significantly to BH4 therapy.

2. Materials and methods

2.1. Patients

This is a single-center, retrospective observational study at Tulane Hayward Genetics Center combining case reports of 85 patients with PKU who were trialed on 20 mg/kg oral BH4 once daily after food from October 2007 to August 2023. This study was approved by the Tulane University Institutional Review Board (IRB). We analyzed the outcome of BH4 treatment on plasma Phe and dietary Phe intake.

2.2. BH4 responsiveness determination

Patients that experienced a decrease in plasma Phe of 30% or greater from historical plasma Phe after being trialed on a pharmacologic dose of BH4 (20 mg/kg/day) were considered BH4 responders. Among BH4 responders, a group of patients had a doubling of dietary Phe tolerance in addition to a 30% or greater decrease in plasma Phe on 20 mg/kg/d of BH4. These patients were considered BH4 “super-responders” for this study.

2.3. Data collection

Data were retrospectively collected from electronic and paper medical records. We reviewed records from 3 months prior to initiation of a patient's BH4 therapy to August 2023. Data collected included birthdate, sex, race, weight, height, body mass index (BMI), genotypes, phenotypes, dietary Phe intake, and plasma Phe levels. Three plasma Phe levels were collected at baseline (5 values prior to initiation of BH4), and after BH4 initiation at 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 3 months, 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years. Mean of Phe value was used for the Phe level of year 2–16. Different methods were used to estimate patients' dietary Phe intake at specific time points, when available, including 24-h diet recalls and self-reported dietary records. As a part of treatment, we attempted to obtain the most accurate picture of actual protein intake from patients through common clinical practices. We did not distinguish high quality protein intake from total protein intake, but most patients avoided high quality protein sources as part of the PKU diet. For the purpose of this study, an unrestricted diet is defined as a diet not limited in dietary protein. Genotypes, phenotypes, and dietary Phe tolerance of BH4 “super responders” were analyzed. A paired one-tail student *t*-test was used to measure statistical significance ($P \leq 0.05$) of pre- and post-BH4 plasma Phe levels and pre- and post-BH4 dietary Phe tolerance increase.

3. Results

3.1. Patient characteristics

Our clinic trialed 85 patients on BH4, and 54 patients were considered BH4 responders (63.5%). Of these 54 patients, the majority of them were white (51/54). African American and other ethnicities were seen in 1 and 2 cases (biracial and Jordanian), respectively. Approximately 61% were female and 39% were male. Of 54 BH4 responders, 21 patients had classical PKU and 29 patients had non-classical PKU (mild PKU or HPA). The phenotype of 4 patients was not classified because untreated plasma Phe levels were unavailable. However, one of four patients had classical-mild PKU and classical PKU-related genotype (c.284_286delTCA and c.842 + 1G > A). Ten patients were pregnant during treatment, and they continued BH4 treatment without complications. At most recent follow-up, 12/54 (22.2%) BH4 responders had discontinued BH4 therapy. Of these 12 patients, seven patients transitioned to pegvaliase treatment due to poor metabolic control due to poor compliance with diet, and 5 patients discontinued BH4 due to poor compliance due to poor compliance with BH4.

Among 54 BH4 responders, 19 patients had a rapid drop in plasma Phe levels and significant increase in their dietary Phe tolerance with at least a 2-fold increase over pre-BH4 Phe tolerance. Eight out of 19 patients had Phe levels >360 μmol/L before starting the BH4 trial, confirming they reached their PHE intake limit. Eleven out of nineteen patients had Phe levels <360 μmol/L before starting the trial. We evaluate Phe tolerance over a patient's lifespan, but not necessarily right before starting BH4. If Phe levels are consistently below mid-treatment range over the past several months, then we often challenge their Phe tolerance. Baseline characteristics of 19 patients from 17 families are presented in Table 1. Fifty-eight percent (14/19) were pediatric at initiation of BH4. Forty-two percent of patients had classical PKU. However, we were not able to classify the phenotype in 1 patient, whose initial plasma Phe levels before PKU treatment were unavailable. Mean age at initiation of BH4 therapy was 10.93 ± 10.67 years. Mean age of duration of BH4 therapy was 10.52± 3.785 years. At most recent visits, all pediatric patients had weight, height, and BMI in the normal range. Among 9 adults, 5/9 were noted to have BMI above the normal range (>25 kg/m^2) including 1 in class 3 obesity range, 1 in class 1 obesity range, 2 in class 2 obesity range and 2 in overweight range.

3.2. Plasma Phe levels during treatment

Plasma Phe values across the study (at baseline, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 2 month, 3 months, 6 months, 8 months, and annual mean for each year after initiation of BH4 therapy, and at most recent follow-up) of 19 patients are shown in Fig. 1. Prior to BH4 therapy, baseline plasma Phe levels were above treatment range (120–360 μmol/L) in 8 patients (42.1%), and within treatment range (120–360 μmol/L) in 11 patients (63.2%). Baseline mean plasma Phe levels ± SD of 19 patients were 370.5 ± 237.2 μmol/L. Of the 19 patients, 8 patients with classical PKU had an average plasma Phe value of 386.0 + 310.0 μmol/L and 10 nonclassical patients had an average plasma Phe value of 363.6 + 146.8 μmol/L. There was no significant difference between the

Table 1
Baseline characteristics of 19 BH4 "super-responders".

Characteristic	Total $N = 19$
Gender, n (%)	
- Male	9 (47.4%)
- Female	10 (52.6%)
Ethnicity, n (%)	
- Caucasian	18 (94.7%)
- African American	0 (0%)
- Jordanian	1 (5.3%)
Phenotype, n (%)	
- Classical PKU	8 (42.1%)
- Non-classical PKU	10 (52.6%)
- Unknown	1 (5.3%)
Age at initiation BH4 therapy, n (%)	
- 1–18 yr	14 (73.7%)
- ≥18–60 yr	5 (26.3%)
Mean age at initiation of BH4 therapy (y) ± SD (min, max)	10.93 ± 10.76 (1.67, 35.42)
Duration of BH4 therapy (y) ± SD (min, max)	10.52 ± 3.85 (3.50, 15.83)
Age at most recent follow-up (y) ± SD (min, max)	21.65 + 12.18 (11.0, 45.0)
- 1–18 yr	10 (52.6%)
- ≥18–60 yr	9 (47.4%)
Most recent growth parameter among pediatric patients (1–18 yr)	
- Weight (mean of percentile ±SD (min, max)	63.8± 29.2 (10, 96)
- Height (mean of percentile ±SD (min, max)	55.7± 35.9 (4, 96.2)
BMI at most recent visit	
- 1–18 yr (mean of percentile ± SD)	62.8 ± 24.8 (29.5, 90.3)
- ≥18–60 yr (mean) ± SD (min, max)	28.9 + 7.8 (22.5, 44.5)

mean plasma Phe values at baseline among the two phenotypes ($P = 0.84$). Eighteen patients had a significant decrease in plasma Phe level at 1 week after initiation of BH4 with the mean ± SD decrease of 61.89 ± 16.6% (min 36.2%, max 92.6%) from baseline. One patient had a plasma Phe of 179 μmol/L at baseline and did not have a drop in plasma Phe level after BH4 therapy. However, we considered this patient a BH4 "super-responder" due to significantly increased dietary Phe tolerance while on BH4 treatment. Plasma Phe levels (mean ± SD) were decreased after BH4 therapy at 1 week, 2 weeks, 1 month, and 1 year at 142.2 ± 88.1, 174.4 ± 87.9, 157.1 ± 84.4, and 283.9 ± 273.0 μmol/L, respectively. Reduction of plasma Phe levels among classical and non-classical PKU were not significantly different after BH4 therapy at 1 week ($P = 0.77$), 2 weeks ($p = 0.78$), 1 month ($P = 0.90$), 6 months (0.09) and 1 year ($P = 0.19$). Plasma Phe (mean ± SD) at most recent follow-up was 326.7 + 185.4 μmol/L. Of the 19 patients, 4 patients (ages 6.3, 12.2, 13.4 and 16.8 years) had the most recent average of 3 plasma Phe values above treatment range, ranging from 441 to 919 μmol/L. Of these four, two patients transitioned to pegvaliase treatment.

3.3. Dietary Phe tolerance across the study

Dietary Phe intake across the study and plasma Phe levels among 19 BH4 "super responders" are shown in Fig. 2. Mean daily dietary Phe tolerance in 19 patients at baseline ± SD was 595.0 + 255.6 mg/day. Nine patients with classical PKU had an average baseline ± SD dietary Phe intake of 545.6 ± 206.7 mg/day, and nine patients with non-classical PKU had an average baseline dietary Phe intake of 621.7 + 308.9 mg/day. There was no significant difference between the mean Phe intake at baseline between the two phenotypes ($P = 0.552$). Mean ± SD of medical food among 19 patients prior to BH4 therapy was 27.7 ± 13.5 g/day (range 0–60 g/day). After initiation of BH4 therapy, six patients were able to increase dietary Phe tolerance at least 2-fold, compared to baseline, at 1 month after initiation of BH4 therapy. Furthermore, 17/19 patients were able to increase Phe tolerance at least 2-fold by 6 months from baseline after initiation of BH4 therapy. Of those 17 patients, 2 patients were on an unrestricted Phe diet after BH4 therapy. Mean dietary Phe tolerance at 1 month, 6 months, 1 year, and most recent follow-up were 1026.4 ± 642.6, 1450.7 ± 689.3, 1448 ± 754, and 2260.3 ± 1414.7 mg/day, respectively. Mean dietary Phe tolerance (mg/day) increased significantly 1.6-fold, 2.7-fold, 2.9-fold, and 3.7-fold at 1 month, 6 months, 1 year, and at most recent follow-up, respectively. Maximal individual Phe tolerance increase at 1 month, 6 months, 1 year, and most recent follow-up was 3.8-fold, 3.8-fold, 4-fold, and 9.6-fold, respectively. Length of treatment for this study (mean ± SD) was 10.8 + 3.8 yr. (Min 3.5, Max 15.8). At 1 year after initiation of BH4 therapy, 2 patients were able to discontinue dietary Phe restriction. At most recent follow-up, 4 patients (21%) no longer required dietary protein restriction. Among these patients, dietary Phe intake was not available for analysis. At most recent follow-up, 6

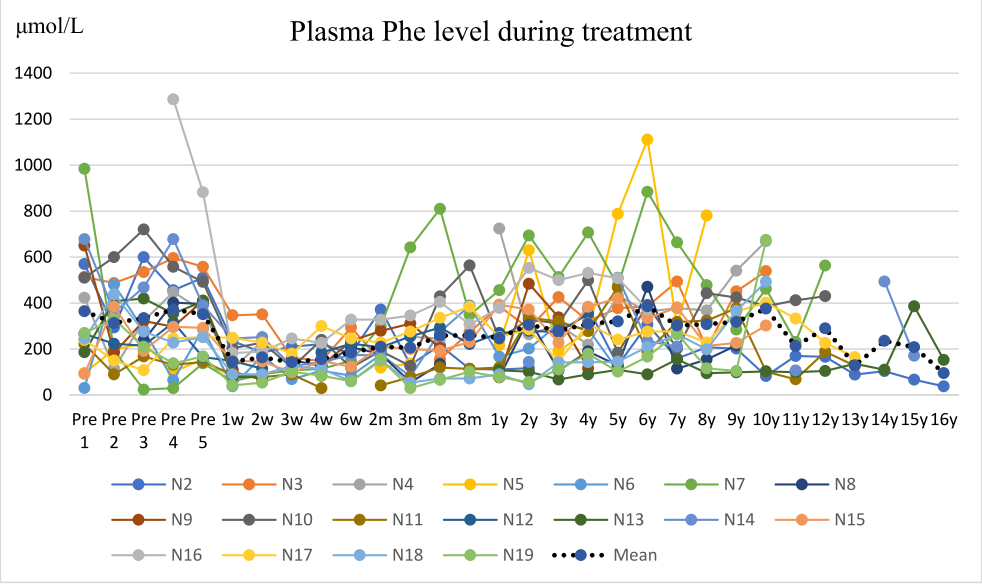

Fig. 1. Plasma Phe level (μmol/L) of 19 patients: 5 levels prior to BH4 treatment, and 1 week, 2 weeks, 4 weeks, 6 weeks, 3 months, 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years after initiation of BH4 treatment.

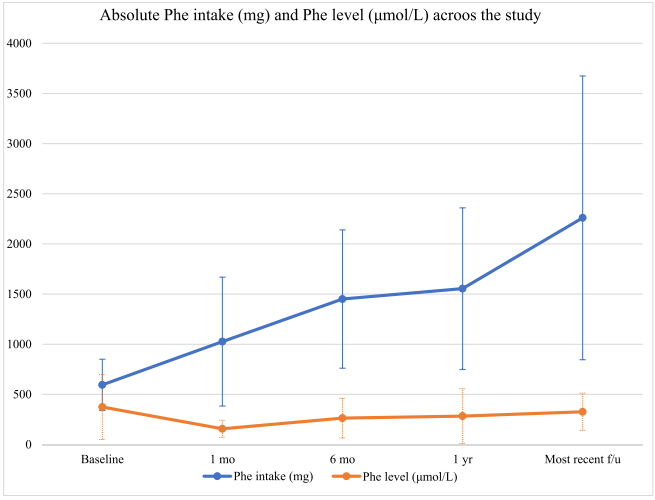

Fig. 2. Absolute dietary Phe intake (mg) and plasma Phe level (μmol/L) with standard error in 19 BH4 "super responders" across the study.

patients no longer required medical food. Mean ± SD of medical food among 13 patients at most recent visit was 23.8 ± 21.3 g/day (range 0–60 g/day).

3.4. Genotypes and phenotypes

Of the 19 patients, mutation analysis for the *PAH* gene was performed in 18 patients from 16 families. Bi-allelic *PAH* mutations were detected in 17 patients. Only one pathogenic variant was detected in one patient. For this particular patient, molecular testing for other hyperphenylalaninemia related genes was also negative. Twenty-one mutations, allele frequency, and predicted residual enzyme activity are shown in Table 2. Frequency of *PAH* variants found in our cohort are diverse. The most common variants were Arg408Trp (5; 2 siblings), Leu48Ser (4), and Tyr414Cys (4; 2 siblings). Residual PAH activity of specific mutations was defined in the PAHvdb database and Himmelreich et al., 2018 [28]. Among 21 *PAH* variants, at least 8 variants are predicted to have residual enzyme activity of ≥25%, which present on 18 alleles of 14 patients. Sixteen out of eighteen patients with molecular results have at least one allele associated with residual PAH activity at ≥25%. Allelic phenotype value (APV) scores correlate well with a patient's phenotype. Seven patients with classic PKU had an APV score < 2.6 for both variants.

4. Discussion

Tetrahydrobiopterin (BH4) has been shown to enhance PAH activity in a subset of PKU patients and has been FDA-approved as an adjunct to dietary therapy. In addition, BH4 may allow responders to maintain plasma Phe within a treatment range while increasing protein intake [24,25,29,30]. Increased dietary Phe tolerance relative to baseline ranges from 1-fold to 5-fold after BH4 therapy [24,29,31,32]. Some patients may either significantly liberalize or unrestrict protein intake in their diet [24]. Few studies have characterized the maximal benefit of oral BH4 on dietary Phe tolerance. Our findings demonstrated a subset of BH4 responsive PKU patients (19/54 responders), who were able to increase dietary Phe tolerance at least 2-fold after initiation of BH4 therapy and maintain their plasma Phe levels in the treatment range. This effect was seen in both classical (42.1%) and non-classical PKU patients (52.6%). One patient was able to increase dietary Phe tolerance 3.8-fold after 1 month of BH4 therapy. Six patients no longer require medical food at their most recent visit. Four out of these six patients had a BH4 response allowing a normal, unrestricted diet at most recent follow-up. This subset of BH4 responders maintained high dietary Phe tolerance with a minimum of 2-fold and maximum of 9.6-fold increase over baseline (mean 3.7-fold) at the most recent follow-up, ranging from 3.5 to 15.8 years.

BH4-responsive genotypes have been characterized for many diverse genotypes [16]. In relation to the genotype among 18 patients, 15 patients carried at least one BH4-responsive variant and/or had at least one mutation predicted in vitro to have residual enzyme activity of >25%. Patient 6 has 2 mutations of unclear BH4-responsive status (p.Ile95del and IVS7 + 1G > A). However, p.Ile95del was predicted in vitro to have PAH enzyme activity of 27%. Six patients were noted to have one BH4-nonresponsive variant on the second allele with BH4-responsive variant. These findings support previously described genotype-phenotype correlation, suggesting that BH4 responsiveness may be anticipated by certain *PAH* phenotypes. Molecular testing may also fail to identify individuals with PKU who have pathogenic variants that are not detected by the standard sequencing method such as deep intronic mutations [33,34]. As seen in our study, a second mutation was not detected in one patient.

The restrictive nature of the PKU diet creates a burden on individuals living with this condition. Some of the reasons patients with PKU are unable to comply with the dietary treatment include poor access to treatment, low education level, lack of financial resources, lack of social support, complex medical systems, and low self-efficacy [35]. Response to BH4 therapy may benefit patients in several ways. Maintenance of blood Phe within a safe treatment range can enhance neuro-cognitive functioning and stabilize labile behavior characteristics of poorly controlled PKU. Additionally, when BH4 therapy supports an increase in dietary Phe intake, many of the barriers to dietary compliance are removed. Nutritional status may be improved when a wider variety of more nutritionally complete foods can be consumed, and reliance on medical food for nutritional support is no longer required [27,36]. This liberalized diet provides better metabolic utilization of micronutrients and amino acids from intact protein.

Although questionnaires assessing quality of life were not administered as a treatment measure in this cohort of patients, improved general quality of life was reported by many patients. Although not quantitated in this study, and treatment bias cannot be ruled out, it is feasible to attribute this self-reported benefit to the primary and secondary treatment effects already mentioned and to consider it when considering BH4 therapy.

This study has some limitations. Methods used for measuring dietary Phe intake which relies on patient recall. Phe intake was not always challenged prior to starting BH4. Due to the retrospective design of this study, we relied on common clinical practices for analyzing Phe intake including 24-h recalls and self-reported dietary records. These methods introduce inaccuracies due to recall bias, inaccurate reports, changes in normal eating patterns, altered consumption from heightened awareness, and difficulties in quantifying protein intake accurately [37]. In addition, complete data on plasma Phe levels and dietary Phe intake was not available for some patients. Lastly, there is some evidence that Phe tolerance increases throughout different stages of life such as puberty and adolescence [38]. Some of the increase in Phe tolerance may not be from sapropterin treatment alone due to not controlling for these life stages. However, most of our patients (17/19) were able to increase their Phe tolerance 2-fold by 6 months which is a short duration.

5. Conclusion

A subset of PKU patients demonstrated marked dietary benefit from oral BH4 treatment while maintaining therapeutic plasma Phe values. Twenty two percent (19/85) of our PKU patients trialed on BH4 had a rapid and significant decrease in blood Phe and an increase in dietary Phe tolerance of 2-fold to 9.6-fold. Four patients were able to assume a completely normal diet. BH4 therapy enabled reported benefits including improvement in quality of life as reported by the patients and

Table 2
Genotype-Phenotype characteristics of Group 1 BH4 responders.

Patient number	Phe at diagnosis (µmol/L)	Age at initiation of BH4 treatment (yr)	Variant 1 (APV)	Variant 2 (APV)	PAH activity[a]	Phe tolerance (mg/d) at baseline	Phe tolerance (mg/d) at most recent follow-up (fold)	Period of BH4 treatment (yr)
1	816	30.4	c.241A>C p.Thr81Pro (ND)	**c.1066-3C>T IVS10-3C>T** (0)	ND ND	750	3000 (4)	10.1
2	396	1.7	c.1222C>T p.Arg408Trp (0)	**c.1241A>G p.Try414Cys** (5.1)	2 28,50,80	300	1000 (3.3)	7
3	720	2	c.1222C>T p.Arg408Trp (0)	**c.1241A>G, p.Try414Cyc** (5.1)	2 28,50,80	300	1000 (3.3)	9.17
4	2760	2.8	**c.143T>C p.Leu48Ser** (2.1)	c.60+5G>T IVS1+5G>T (0)	47 ND	440	1076 (2.45)	7.25
5	2298	4.5	**c.194T>C p.Ile65Thr** (1.4)	c.1045T>C p.Ser349Pro (0)	26,27,33,480	325	650 (2)	10
6	ND	22.2	c.284_286delTCA p.Ile95del (2.2)	c.842+1G>A IVS7+1G>A (0)	27 ND	900	2500 (2.8)	4.7
7	1332	13.1	**c.1042C>G p.Leu348Val** (1.6)	c.912+1G>A IVS8+1G>A (0)	25,33,38 ND	385	1050 (2.7)	13.7
8	1794	5.1	**c.117C>G p.Phe39Leu** (1)	**c.117C>G, p.Phe39Leu** (1)	73 73	560	1700 (3)	10.7
9	2520	6.4	c.1222C>T p.Arg408Trp (0)	**c.143T>C p.Leu48Ser** (2.1)	2 47	735	Unrestricted 3900 (5.3)	13.2
10	1104	19.4	c.1055delG P.Gly352ValfsTer48 (0)	**c.529G>C p.Val177Leu** (7.5)	ND ND	525	Unrestricted 5050 (9.6)	13.6
11	1560	8.25	c.1222C>T (p.Arg408Trp) (0)	**c.143T>C p.Leu48Ser** (2.1)	2 47	500	1220 (2.4)	6.8
12	1286	35.4	**c.143T>C p.Leu48Ser** (2.1)	c.553_706+647del (ND)	47 ND	1000	2800 (2.8)	5.9
13	312	1.9	**c.1241A>G p.Tyr414Cyc** (5.1)	**c.204A>T p.Arg68Ser** (5.4)	28,50,80 25	750	Unrestricted 4500 (6)	9.3
14	468	1.9	**c.1241A>G p.Tyr414Cyc** (5.1)	**c.204A>T p.Arg68Ser** (5.4)	28,50,80 25	750	Unrestricted 4500 (6)	9.3
15	2412	5	c.1066-11G>A IVS10-11G>A (0)	Not detected	5	440	965 (2.2)	10.6
16	650	5.25	Not available	Not available	ND	345	750 (2.2)	4.5
17	474	7.6	c.1222C>T p.Arg408Trp (0)	c.1357delTAAG p.*453Proext*33 (0)	2 ND	700	3000 (4.3)	1.3
18	604	1.83	**c.117C>G p.Phe39Leu** (1)	**c.117C>G p.Phe39Leu** (1)	73 73	600	2000 (3.3)	2.3
19	ND	29	**c.631C>A p.Pro211Thr** (9.3)	c.754C>T p.Arg252Trp (0)	72 0, 15	800	3000 (3.75)	15.8

Mutations in bold are defined as BH4 responsive, mutations highlighted in red are defined as BH4-nonresponsive mutations [16].
ND, no data.
Allelic phenotype value (APV): APV is a tool to investigate genotype-phenotype associations [Garbade SF et al., 2018]. APV = 0–2.7 classic PKU variants; APV = 2.8–6.6 mild PKU variants; APV = 6.7–10.0 mild HPA variants.
[a] Predicted residual enzyme activity of variant 1 (upper row) and variant 2 (lower row) (BIOPKU database, www.biopku.org and Himmelreich 2018).

nutritional status while maintaining Phe level in treatment range.

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Author contributions

JU, KC, GN, AC, CC and HCA designed and conceptualized the study and performed clinical analysis. JU, KC and GN performed data curation. YL, MM, MC performed molecular testing and analysis. JU drafted the manuscript. All authors were involved with revising the manuscript.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Tulane University Institutional Review Board (IRB).

CRediT authorship contribution statement

Jariya Upadia: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Kea Crivelly:** Data curation, Formal analysis, Writing – review & editing. **Grace Noh:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Amy Cunningham:** Writing – review & editing. **Caroline Cerminaro:** Writing – review & editing. **Yuwen Li:** Formal analysis, Investigation, Writing – review & editing. **Meredith Mckoin:** Investigation, Methodology, Resources. **Madeline Chenevert:** Investigation, Methodology, Resources. **Hans C. Andersson:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

The data presented in this study are available on request from the corresponding author.

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