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Recommended Citation

Hawks, Zoe; Shimony, Joshua; Rutlin, Jerrel; Grange, Dorothy K.; Christ, Shawn E.; and White, Desiree A., "Pretreatment cognitive and neural differences between sapropterin dihydrochloride responders and non-responders with phenylketonuria." *Molecular Genetics and Metabolism Reports*. 12, 8-13. (2017). https://digitalcommons.wustl.edu/open_access_pubs/5664

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Pretreatment cognitive and neural differences between sapropterin dihydrochloride responders and non-responders with phenylketonuria



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ARTICLE INFO

Article history:

Received 16 December 2016

Accepted 28 January 2017

Available online xxxx

Keywords:

Phenylketonuria

Sapropterin dihydrochloride

BH₄

Intelligence

IQ

White matter

Diffusion tensor imaging

ABSTRACT

Sapropterin dihydrochloride (BH₄) reduces phenylalanine (Phe) levels and improves white matter integrity in a subset of individuals with phenylketonuria (PKU) known as “responders.” Although prior research has identified biochemical and genotypic differences between BH₄ responders and non-responders, cognitive and neural differences remain largely unexplored. To this end, we compared intelligence and white matter integrity prior to treatment with BH₄ in 13 subsequent BH₄ responders with PKU, 16 subsequent BH₄ non-responders with PKU, and 12 healthy controls. Results indicated poorer intelligence and white matter integrity in non-responders compared to responders prior to treatment. In addition, poorer white matter integrity was associated with greater variability in Phe across the lifetime in non-responders but not in responders. These results underscore the importance of considering PKU as a multi-faceted, multi-dimensional disorder and point to the need for additional research to delineate characteristics that predict response to treatment with BH₄.

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1. Introduction

Phenylketonuria (PKU) is a recessive hereditary disorder characterized by deficient or absent phenylalanine hydroxylase (PAH). Consequently, catalysis of phenylalanine (Phe) is disrupted, and concentrations of blood Phe are elevated [36]. If untreated, PKU may lead to profound neurologic problems and intellectual disability [31,32].

In recent decades, the most severe of these neural and cognitive consequences have been mitigated by newborn screening programs that identify infants with PKU and implement dietary treatment to limit Phe intake. Nevertheless, individuals with early- and continuously-treated PKU exhibit brain abnormalities [2,3,8,11], score slightly lower than expected on tests of intelligence [35], and are at increased risk for executive (for review, see [10]), psychosocial, and psychiatric difficulties [9,20,43].

Dietary restriction of Phe-containing foods remains the most commonly prescribed treatment for PKU, but emerging treatments hold promise for the future [40,45]. One promising pharmaceutical treatment is a synthetic form of tetrahydrobiopterin (BH₄), sapropterin dihydrochloride. BH₄ is a cofactor for PAH, facilitating the conversion of Phe into tyrosine and thereby reducing blood Phe in a subset of individuals with PKU who have residual PAH activity [30].

Although there is some debate regarding the degree to which blood Phe must be reduced to consider an individual a responder to BH₄ [38, 46], responsiveness is often defined as a reduction in Phe \geq 30% compared to a pretreatment baseline [6]. In the present study, BH₄ responders exhibited average reductions in Phe \geq 30% over the course of 4 weeks of treatment. Previous studies using a similar reduction criterion found that 38–54% of individuals with PKU responded to BH₄ within 8 h of administration [18,19], 46–52% responded within 24 h of administration [18,19], and 50% responded within 48 h of administration [19].

Factors distinguishing BH₄ responders from non-responders are not well understood. Although evidence suggests that individuals with mild PKU respond better than those with classic PKU [26], a subset of individuals with classic PKU nonetheless respond to the drug [21,29]. In addition, biochemical [22,25] and genetic [22,26,34] variables may differ between responders and non-responders. For example, in the biochemical domain, Humphrey et al. [25] found that non-responders typically have greater variability in blood Phe and a higher Phe to tyrosine ratio than responders. In the genetic domain, Karačić et al. [26] linked BH₄ responsiveness to mutations on the 12q22–24 chromosome of the gene encoding PAH.

In contrast with the proliferation of research on biochemical and genetic differences between BH₄ responders and non-responders, cognitive and neural differences remain largely unexplored. The present study aimed to address this gap in knowledge. To do so, we examined data related to indices of Phe control, microstructural white matter integrity, and intelligence that were collected prior to BH₄ administration

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in individuals who were subsequently identified as BH₄ responders and non-responders. To provide a normative context for results, comparisons were also made with a healthy non-PKU control group.

2. Material and methods

2.1. Participants

Individuals with classic PKU ($n = 29$; 18 males, 11 females) were recruited through metabolic clinics at St. Louis Children's Hospital ($n = 11$), University of Missouri ($n = 7$), University of Florida ($n = 4$), St. Louis University ($n = 3$), Washington University ($n = 2$), New York Medical College ($n = 1$), and University of Nebraska ($n = 1$), with all cognitive assessment and neuroimaging procedures conducted at either Washington University ($n = 19$) or University of Missouri ($n = 10$). All individuals were diagnosed in early infancy and thereafter placed on continuous dietary treatment to limit Phe intake. No individual had a history of major medical, psychiatric, or learning disorder unrelated to PKU.

Baseline cognitive, neuroimaging, and blood Phe data were obtained from participants prior to treatment with BH₄ (20 mg/kg/day). During the 4 weeks of screening for response to BH₄ that followed, blood Phe was monitored weekly, and participants were instructed to maintain their usual diets. Phe levels recorded in the year preceding treatment were averaged to calculate baseline Phe, and Phe levels from the 4-week screening period were averaged to calculate percent reduction in blood Phe relative to baseline Phe. On this basis, individuals were classified as responders or non-responders to BH₄ (Phe data are reported in Table 3; secondary analyses were also run using lifetime mean Phe as the baseline metric against which screening Phe was compared, and results were consistent). Responders ($n = 13$; 8 males, 5 females) exhibited a reduction in Phe $\geq 30\%$, whereas non-responders ($n = 16$; 10 males, 6 females) failed to exhibit such change.

Age ranged from 9–35 years ($M = 18.8$, $SD = 9.4$) for responders and 8–33 years ($M = 16.6$, $SD = 8.1$) for non-responders. Pertaining to race/ethnicity, 8% of responders and 0% of non-responders identified as members of a minority group. There were no significant differences between responders and non-responders in age, gender, or race/ethnicity ($p > 0.05$ in all instances).

Baseline cognitive and neuroimaging data from individuals with PKU were also compared with those of healthy controls ($n = 12$; 8 males, 4 females) recruited from the St. Louis community. Within the control group, age ranged from 7–33 years ($M = 17.8$, $SD = 8.0$), and 8% identified as members of a minority group. No control reported a history of major medical, psychiatric, or learning disorder, and there were no significant differences between individuals with PKU and controls in age, gender, or race/ethnicity ($p > 0.05$ in all instances).

2.2. Procedures

Data included in this report are components of a longitudinal study exploring the effects of BH₄ on biochemical, neural, and cognitive outcomes in individuals with early- and continuously-treated PKU. Approval for this study was obtained from institutional review boards at Washington University and University of Missouri, the sites at which all cognitive and neuroimaging data were collected. Participants and/or their guardians provided written informed consent prior to enrolling in the study, and the cognitive and neuroimaging components of the study were typically completed in a single session lasting 4 h. Previous manuscripts have reported data from the longitudinal dataset to explore differences in cognition and white matter integrity between PKU and control groups¹; however, pretreatment cognitive and neural

differences between responders and non-responders have not been examined previously.

2.3. Phenylalanine

We evaluated 6 indices of Phe control. Of these indices, 3 were related to implementation of treatment with BH₄ (baseline Phe, screening Phe, percent reduction in Phe) and 3 were related to Phe control over the lifetime prior to treatment with BH₄ (mean Phe, SD Phe as an indicator of variability, index of dietary control [IDC]). Baseline and screening Phe were used to calculate percent reduction in Phe, thereby determining group status (i.e., responder, non-responder). Lifetime mean Phe and SD Phe were examined because they have been negatively associated with cognitive performance and white matter integrity [23,24,41] and are used with greatest ease in metabolic clinics. The IDC was examined to control for the fact that fewer Phe levels are typically obtained as individuals with PKU age; this index was computed as the mean of each individual's annual median Phe level, and thus provided a weighted average of lifetime Phe.

2.4. Intelligence

IQ was estimated using a composite based on standard scores (standard score normative mean = 100, SD = 15) from the Matrix Reasoning and Vocabulary subtests of the Wechsler Abbreviated Scale of Intelligence (WASI; [42]). The two subtest scores were also examined separately because they provided estimates of performance and verbal IQ, respectively. Administration and scoring followed test manual instructions.

2.5. White matter integrity

Neuroimaging procedures are described in detail by Antenor-Dorsey et al. [3]. Briefly, scans were run on a Siemens TIM Trio 3.0 T imaging system (Erlangen, Germany). Diffusion tensor imaging (DTI) data reflecting microstructural white matter integrity were collected using an echo planar imaging (EPI) sequence along 25 non-collinear diffusion gradients [TR = 12,437 (Washington University; WU) and 9900 (University of Missouri; UM), TE = 102 (WU and UM), flip angle = 90° (WU and UM), FOV = 864 × 864 (WU) and 768 × 768 (UM), voxel resolution = 2.0 × 2.0 × 2.0 (WU and UM)]. Diffusion weighted images were registered to weighted structural images and then to an in-house atlas at Washington University. Parametric maps were subsequently generated for mean diffusivity (MD), the DTI component of interest in this study.

MD was compared across study groups using region of interest (ROI) and tract based spatial statistics (TBSS) analyses (FSL software, Oxford, UK; [39]). We did not control for age because groups were statistically equivalent on this metric, and previous analyses of DTI and cognition did not indicate significant interactions between age and group [3]. As in our previous studies [3,23,44], we focused on the following 10 white matter ROIs to provide a broad sampling across a range of brain regions: hippocampus, putamen, prefrontal cortex, optic radiation, posterior parietal-occipital, centrum semiovale, thalamus, and corpus callosum (genu, body, splenium). MD data from two non-responders were unavailable due to MRI contraindications and movement artifact, but this did not result in between-group demographic differences.

2.6. Statistical analyses

As a starting point, to minimize the number of group-wise (responder, non-responder, control) comparisons and thereby reduce Type I error rate, omnibus ANOVAs were conducted to examine the effect of group (responder, non-responder, control) on each intelligence and MD variable. When significant ANOVA results were obtained, one-tailed independent samples *t*-tests were conducted to examine the effect of group on

¹ Sample size may differ slightly across studies due to variations in exclusion criteria. The present study excluded participants if response to BH₄ was unknown, IQ data were unavailable, or age was <7 years at baseline.

Table 1

Omnibus results comparing intelligence and MD among responders, non-responders, and controls.

	Statistical findings		
	F	p	ω^2
Intelligence			
IQ	4.69	0.02	0.15*
Vocabulary	0.91	0.41	<0.01
Matrix reasoning	7.11	<0.01	0.23*
ROI			
Hippocampus	5.82	<0.01	0.20*
Putamen	5.63	<0.01	0.19*
Prefrontal	8.50	<0.01	0.28*
Optic radiation	12.43	<0.01	0.37*
Parietal-occipital	18.16	<0.01	0.47*
Centrum semiovale	10.73	<0.01	0.33*
Thalamus	1.19	0.32	<0.01
Genu of CC	15.81	<0.01	0.43*
Body of CC	9.01	<0.01	0.29*
Splenium of the CC	3.37	<0.05	0.11*

Note: asterisks denote statistically significant results; F = variance between groups/variance within groups; ω^2 = omega-squared; degrees of freedom = (2, 38) for intelligence variables and (2, 36) for ROIs.

each relevant intelligence and MD variable. Of particular interest were differences between responders and non-responders. *t*-tests were also used to explore differences in Phe indices between responders and non-responders.

To avoid masking novel results that may drive future research, we did not control for multiple comparisons and set our alpha level at 0.10. However, to maintain statistical rigor, we considered results significant only when *p*-values less than our alpha threshold were associated with medium or large effect sizes. To adjust for bias due to small sample size, Hedge's *g* and omega-squared (ω^2) were used as measures of effect size for *t*-test and ANOVA comparisons, respectively. Consistent with conventions, $g = 0.5$ and $\omega^2 = 0.06$ designated medium effect sizes, and $g = 0.8$ and $\omega^2 = 0.14$ designated large effect sizes [13,27]. A growing body of research supports our statistical approach, emphasizing the pitfalls of null hypothesis significant testing [14,16,17,28] and the importance of effect sizes and confidence intervals [4,15,37].

3. Results

3.1. Omnibus results

As shown in Table 1, omnibus ANOVA results revealed significant effects of group on IQ and Matrix Reasoning. With respect to MD, there

Table 2

Mean (SD) and statistical findings comparing controls vs. responders and controls vs. non-responders.

	Control	Responders	Non-responders	Statistical findings							
				Control vs. Responders				Control vs. Non-responders			
				t	p	Hedge's <i>g</i>	95% CI	t	p	Hedge's <i>g</i>	95% CI
Intelligence											
IQ	108.5 (10.6)	104.2 (10.9)	95.6 (12.5)	1.01	0.16	0.39	−3.02	2.96	<0.01	1.07*	5.48
Matrix reasoning	113.4 (9.9)	104.4 (11.4)	97.6 (11.4)	2.12	0.02	0.81*	1.12	3.92	<0.01	1.42*	5.95
ROI											
Hippocampus	0.84 (0.02)	0.87 (0.08)	0.79 (0.06)	1.24	0.88	0.48	−0.07	2.91	<0.01	1.05*	0.02
Putamen	0.71 (0.03)	0.69 (0.04)	0.66 (0.05)	1.90	0.04	0.74*	0.00	3.47	<0.01	1.26*	0.03
Prefrontal	0.74 (0.05)	0.72 (0.03)	0.67 (0.05)	1.38	0.09	0.53*	>−0.01	3.64	<0.01	1.38*	0.04
Optic radiation	0.84 (0.04)	0.79 (0.08)	0.70 (0.08)	1.72	0.05	0.67*	>−0.01	5.59	<0.01	2.01*	0.09
Parietal-occipital	0.84 (0.05)	0.75 (0.08)	0.65 (0.09)	3.45	<0.01	1.34*	0.04	6.18	<0.01	2.26*	0.13
Centrum Semiovale	0.73 (0.03)	0.68 (0.07)	0.61 (0.08)	2.31	0.02	0.90*	0.01	5.3	<0.01	1.89*	0.08
Genu of CC	0.83 (0.09)	0.71 (0.08)	0.64 (0.09)	3.59	<0.01	1.39*	0.07	5.23	<0.01	2.02*	0.13
Body of CC	0.85 (0.06)	0.82 (0.09)	0.73 (0.06)	0.99	0.17	0.38	−0.02	4.75	<0.01	1.80*	0.07
Splenium of CC	0.69 (0.05)	0.71 (0.08)	0.64 (0.07)	0.85	0.80	0.33	−0.07	1.95	0.03	0.72*	0.01

Notes: asterisks indicate statistically significant between-group difference; upper bound of all one-tailed confidence intervals is infinity.

Table 3

Mean (SD) and statistical findings comparing indices of Phe control in responders and non-responders.

Phe (μmol/L)	Responders	Non-responders	Statistical findings			
			t	p	Hedge's <i>g</i>	95% CI
Baseline						
Mean (SD)	588.3 (391.0)	746.2 (353.4)	1.13	0.14	0.41	−81.2
Range	135.5–1598.3	179.8–1271.3				
Screening						
Mean (SD)	254.4 (181.9)	789.6 (415.4)	4.64	<0.01	1.56*	336.7
Range	69.5–741.6	184.5–1541.8				
% Reduction						
Mean (SD)	53.0 (16.1)	−6.7 (27.0)	7.38	<0.01	2.55*	45.9
Range	32.2–87.4	−71.6–16.6				
Lifetime Mean						
Mean (SD)	506.8 (262.9)	566.3 (243.2)	0.63	0.27	0.23	−102.7
Range	250.3–1033.7	179.8–1093.2				
Lifetime SD						
Mean (SD)	213.1 (92.7)	285.2 (145.4)	1.62	0.06	0.56*	−3.85
Range	52.8–343.3	91.3–700.4				
IDC						
Mean (SD)	506.2 (240.8)	634.0 (316.9)	1.23	0.11	0.46	−48.7
Range	236.6–1000.2	205.4–1435.8				

Notes: asterisks indicate statistically significant between-group difference; upper bound of all one-tailed confidence intervals is infinity.

were significant effects of group in 9 of 10 white matter ROIs: the hippocampus, putamen, prefrontal cortex, optic radiation, posterior parietal-occipital, centrum semiovale, and genu, body, and splenium of the corpus callosum. The effect of group on Vocabulary was not significant, nor was the effect of group on MD in the thalamus; as such, these variables were excluded from further analyses.

3.2. Control group comparisons

Although not the central focus of our study, to provide a normative context, we evaluated differences in intelligence and MD between controls and each of our PKU groups (see Table 2). Regarding intelligence, IQ was significantly poorer for non-responders than controls. In addition, Matrix Reasoning performance was significantly poorer for both responders and non-responders than controls. In terms of white matter integrity results, MD was significantly lower for both responders and non-responders than controls in the putamen, prefrontal cortex, optic radiation, posterior parietal-occipital, centrum semiovale, and genu of the corpus callosum. MD was also significantly lower for non-responders than controls in the hippocampus and body and splenium of the corpus callosum. Thus, there were significant findings

Table 4
Mean (SD) and statistical findings comparing intelligence and MD in responders and non-responders.

	Responders	Non-responders	Statistical findings			
			t	p	Hedge's g	95% CI
Intelligence						
IQ	104.2 (10.9)	95.6 (12.5)	1.98	0.03	0.71*	1.2
Matrix reasoning	104.4 (11.4)	97.6 (11.4)	1.60	0.06	0.58*	−0.4
ROI						
Hippocampus	0.87 (0.08)	0.79 (0.06)	2.83	<0.01	1.07*	0.03
Putamen	0.69 (0.04)	0.66 (0.05)	1.63	0.06	0.60*	>−0.01
Prefrontal	0.72 (0.03)	0.67 (0.05)	2.89	<0.01	1.06*	0.02
Optic radiation	0.79 (0.08)	0.70 (0.08)	2.92	<0.01	1.09*	0.04
Parietal-occipital	0.75 (0.08)	0.65 (.09)	2.87	<0.01	1.06*	0.04
Centrum semiovale	0.68 (0.07)	0.61 (0.08)	2.30	0.02	0.86*	0.02
Genu of CC	0.71 (0.08)	0.64 (0.09)	2.11	0.02	0.78*	0.01
Body of CC	0.82 (0.09)	0.73 (0.06)	2.83	<0.01	1.07*	0.03
Splenium of CC	0.71 (0.08)	0.64 (0.07)	2.29	0.02	0.86*	0.02

Notes: asterisks indicate statistically significant between-group difference; upper bound of all one-tailed confidence intervals is infinity.

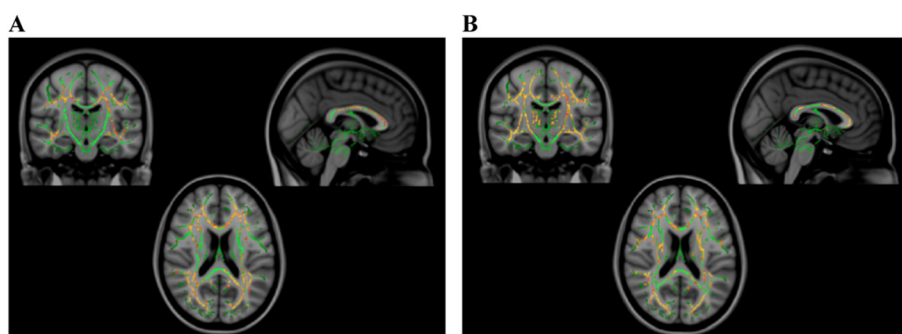


Fig. 1. TBSS findings indicating (A) significantly lower MD in non-responders than responders and (B) a significant inverse correlation between MD and SD Phe in non-responders. Notes: green = TBSS skeleton; red = $p < 0.05$, orange = $p < 0.03$, and yellow = $p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in 6 of 9 ROIs for responders and all 9 ROIs for non-responders, replicating findings from prior studies demonstrating poorer cognition and white matter integrity in individuals with PKU relative to controls (e.g., [3,44]).

3.3. Responder and non-responder comparisons

3.3.1. Phenylalanine

Group differences in baseline Phe, screening Phe, percent reduction in Phe, lifetime mean Phe, lifetime SD Phe, and IDC were evaluated between responders and non-responders (see Table 3). Baseline Phe was not significantly different between responders and non-responders, although in absolute terms baseline Phe was higher for non-responders. As expected given their central roles in determining group membership, significant differences in screening Phe and percent reduction in Phe indicated that blood Phe levels were reduced to a greater extent for responders than non-responders following implementation of treatment with BH₄. A significant difference in SD Phe also emerged, indicating that variability in Phe over the lifetime was greater for non-responders than responders. In contrast, there were no significant differences in either mean Phe over the lifetime or IDC.

3.3.2. Intelligence

Given significant ANOVA results, possible between-group differences in IQ and Matrix Reasoning were assessed in responders and non-responders (see Table 4). In both comparisons, non-responders scored significantly poorer than responders. To situate these results within the context of the single identified group difference in Phe

control, we examined correlations between intelligence and SD Phe in responder and non-responder groups; no results were significant.²

3.3.3. White matter integrity

Group differences in MD between responders and non-responders were similarly evaluated in ROIs for which omnibus results were significant (see Table 4). Significant results emerged in all 9 ROIs (hippocampus, putamen, prefrontal cortex, optic radiation, posterior parietal-occipital, centrum semiovale, and genu, body and splenium of the corpus callosum), with lower MD for non-responders than responders. Irrespective of statistical significance, the chance odds that MD emerges numerically lower (and therefore farther from the value of controls) for non-responders than responders across all 9 ROIs is 0.0020.

Consistent with our ROI findings, TBSS analyses revealed widespread differences in MD (see Fig. 1A), with lower MD for non-responders than responders. We also examined white matter integrity in relation to SD Phe over the lifetime and found that higher SD Phe was associated with lower MD for non-responders (see Fig. 1B) but not responders.

4. Discussion

Previous studies have identified biochemical and genotypic differences between BH₄ responders and non-responders with PKU [22,25, 26,34]. However, very little research has sought to identify cognitive

² Baseline Phe, mean Phe, and IDC were not included in correlational analyses because they did not differ significantly between responders and non-responders; screening Phe and percent reduction in Phe were not included because they determined group membership.

and neural differences between responders and non-responders. The present study aimed to address this gap in the literature by investigating baseline (i.e., prior to treatment with BH₄) differences in indices of Phe control, intelligence, and white matter integrity between individuals with PKU who were subsequently identified as BH₄ responders and non-responders.

To situate our results within the context of previous research, a majority of which investigated cognitive performance and white matter integrity in individuals with PKU relative to controls [3,5,12], analyses were conducted separately comparing intelligence and MD in responders and non-responders relative to controls. Consistent with prior research [2,3,33,35], results indicated that individuals with PKU, regardless of response to BH₄, exhibited poorer intelligence and white matter integrity than controls.

Turning to our central focus, we evaluated group differences between responders and non-responders. With respect to Phe, screening Phe and lifetime SD Phe were significantly higher for non-responders than responders, whereas percent reduction in Phe was significantly lower; there were no significant differences in baseline Phe, lifetime mean Phe, or IDC prior to treatment. Because screening Phe and percent reduction in Phe determined group status, differences between responders and non-responders were expected. The remaining indices of Phe control can be classified as quantifiers of variability in Phe (SD Phe) or Phe concentration (baseline Phe, mean Phe, IDC). As such, Phe variability but not concentration distinguished responders from non-responders, positioning SD Phe as a potential predictor of response to BH₄.

With respect to intelligence, significant differences in IQ and Matrix Reasoning standard scores were observed, with poorer scores for non-responders than responders. A similar pattern emerged when neuroimaging data were examined, reflecting poorer white matter integrity for non-responders than responders prior to treatment with BH₄. Specifically, MD was numerically lower for non-responders than responders in all 9 ROIs submitted to post-hoc analyses (the hippocampus, putamen, prefrontal cortex, optic radiation, posterior parietal-occipital, centrum semiovale, and genu, body, and splenium of the corpus callosum).

Corroborating ROI findings, TBSS analyses revealed that significantly poorer white matter integrity for non-responders than responders was widespread. Additionally, TBSS analyses revealed significant inverse correlations between MD and SD Phe in non-responders but not responders, again pointing to the possible predictive utility of SD Phe.

Collectively, results from our study indicate that BH₄ non-responders differ from responders on the basis of variability in Phe over the lifetime and a number of variables reflecting intelligence and white matter integrity. It is possible that more fine-grained estimates of effect size and/or additional significant differences in Phe, intelligence, and white matter integrity might have been identified in a larger sample. Because PKU is a rare disorder affecting <0.01% of the United States population [7], recruitment of large samples is inherently difficult, but through collaborative efforts future studies might address our sample size limitation. In turn, in conjunction with genotypic data, Phe, intelligence, and white matter integrity data might be used to develop a powerful predictive model of response to BH₄ in a larger sample of individuals with PKU.

Conflict of interest

This study was funded by an Investigator Sponsored Trial award from BioMarin Pharmaceutical Inc. Drs. White, Grange, and Christ have served as consultants and received research funding from BioMarin Pharmaceutical Inc. The content of the article has not been influenced by these relationships.

Acknowledgments

This research was supported by an Investigator Sponsored Trial award from BioMarin Pharmaceutical Inc. (BMRN/40716) and the

Intellectual and Developmental Disabilities Research Center at Washington University with funding from the National Institute of Child Health and Human Development (U54HD087011). The authors wish to thank those who participated in our study for their contributions. We also thank Suzin Blankenship, Elizabeth Toolan, and Laurie Sprietsma for their contributions to study management, as well as the physicians, faculty, and staff of Washington University, University of Missouri, University of Florida, St. Louis University, New York Medical College, and University of Nebraska who generously contributed through recruitment and phenylalanine monitoring.

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