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

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ABSTRACT

Sapropterin dihydrochloride (BH_4) 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 BH_4 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 BH_4 in 13 subsequent BH_4 responders with PKU, 16 subsequent BH_4 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 BH_4.

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 (BH_4), sapropterin dihydrochloride. BH_4 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 BH_4 [38,46], responsiveness is often defined as a reduction in Phe ≥ 30% compared to a pretreatment baseline [6]. In the present study, BH_4 responders exhibited average reductions in Phe ≥ 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 BH_4 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 BH_4 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 BH_4 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 BH_4 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 BH_4 administration

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in individuals who were subsequently identified as BH_4 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 BH_4 (20 mg/kg/day). During the 4 weeks of screening for response to BH_4 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 BH_4 (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 ≥ 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 BH_4 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 groups[1]; 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 BH_4 (baseline Phe, screening Phe, percent reduction in Phe) and 3 were related to Phe control over the lifetime prior to treatment with BH_4 (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 BH_4 was unknown, IQ data were unavailable, or age was <7 years at baseline.