Brain white matter integrity mediates the relationship between phenylalanine control and executive abilities in children with phenylketonuria

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Abstract

We tested the hypothesis that brain white matter integrity mediates the relationship between phenylalanine (Phe) control and executive abilities in children with phenylketonuria (PKU; N=36). To do so, we examined mean diffusivity (MD) from diffusion tensor imaging (DTI) in two white matter brain regions (posterior parietal–occipital, PPO; centrum semiovale, CSO) and lifetime phenylalanine (Phe) exposure; the executive abilities examined included verbal strategic processing, nonverbal strategic processing, and working memory. Mediation modeling showed that MD in the PPO and CSO mediated the relationship between Phe exposure and nonverbal strategic processing, MD in the CSO mediated the relationship between Phe exposure and verbal strategic processing, and MD in the PPO mediated the relationship between Phe exposure and working memory. These exploratory findings demonstrate the importance of using sophisticated modeling procedures to understand the interplay among metabolic control, neural factors, and functional outcomes in individuals with PKU.

Keywords: phenylketonuria; brain; white matter; executive abilities; neuroimaging; mediation

Compliance with Ethics Guidelines

Conflict of Interest

Anna Hood, Jerrel Rutlin, and Joshua Shimony declare that they have no conflict of interest. Desiree White and Dorothy Grange have received research grants from BioMarin Pharmaceutical Inc. and have served as consultants for BioMarin Pharmaceutical Inc.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients included in the study.

Contribution of Authors

Desiree White designed the study, wrote the protocol, trained research staff, supervised data collection, interpreted data, and co-wrote the manuscript. Anna Hood conducted literature review, analyzed and interpreted data, and co-wrote the manuscript. Jerrel Rutlin analyzed and interpreted data and provided input on the writing of the paper. Joshua Shimony contributed to study design and provided statistical analysis, neuroimaging consultation, and input on the writing of the paper. Dorothy Grange contributed to study design, participant recruitment, and provided input on the writing of the paper. All authors contributed to and have approved the final manuscript.

Introduction

Phenylketonuria (PKU; 261600) is an inherited metabolic disorder associated with a deficiency in or absence of the phenylalanine hydroxylase enzyme (EC 1.14.16.1). As a consequence, the amino acid phenylalanine (Phe) is improperly metabolized, which leads to higher than normal Phe levels (De Groot et al., 2010). Although serious cognitive sequelae are generally avoided through early detection and dietary treatment to limit Phe intake (Mitchell et al., 2011; Paine, 1957), individuals with early- and continuously-treated PKU often have lower than expected intellectual abilities (Waisbren et al., 2007), as well as impairments in processing speed (Janos et al., 2012) and executive abilities (Christ et al., 2010; DeRoche & Welsh, 2008).

For decades it has been hypothesized that PKU-related cognitive impairment is associated with dopamine deficiency (De Groot et al., 2010), because elevations in Phe disrupt the neurochemical cascade by which Phe is converted to tyrosine, a precursor of dopamine and other catecholaminergic neurotransmitters (Scriver, 2007). Compromised white matter integrity in the brain, however, is another possible mechanism underlying PKU-related cognitive impairment and represents the focus of the current study. Recent research using diffusion tensor imaging (DTI) indicates that white matter compromise is widespread throughout the brain in individuals with early- and continuously-treated PKU (Antenor-Dorsey et al., 2013; Hood et al., 2014). More specifically, although fractional anisotropy (FA; reflecting the degree of water diffusion asymmetry) is relatively normal, mean diffusivity (MD; reflecting degree of displacement of water molecules) is significantly decreased (Antenor-Dorsey et al., 2013; Scarabino et al., 2009; White et al., 2010, 2013).

In terms of relationships between white matter integrity, cognition, and Phe control in

individuals with PKU, a number of studies have shown that higher Phe is associated with both decreases in MD (e.g., Vermathen et al., 2007; Hood et al., 2014) and poorer cognition (e.g., Christ et al., 2010; Weglage et al., 2013). Our knowledge of relationships between DTI findings and cognition is quite limited, but the three relevant studies to date point to associations between MD across a range of brain regions and IQ (Peng et al., 2004; Antenor-Dorsey et al., 2013; Peng et al., 2013). There is, however, no research in which the interplay among white matter integrity, cognition, and Phe control has been modeled within a single study. The purpose of this exploratory study was to address this substantial gap in our knowledge of PKU. Specifically, we tested the hypothesis that white matter integrity mediates the relationship between Phe control and executive abilities.

Material and Methods

Participants

Children with PKU (n = 36; 19 male, 17 female) were diagnosed soon after birth and received early dietary management to limit Phe intake. Lifetime Phe levels, with gaps of no more than 2 years prior to neuroimaging and cognitive evaluation, were available for all children and ranged from 0 – 2742 μmol/L (*M* = 371.1, *SD* = 282.5). Age ranged from 6 to 18 years (M = 12.2, SD = 3.8), education ranged from 0 to 13 years (M = 6.4, SD = 3.8), and IQ ranged from 75 to 122 (M = 102.1, SD = 10.9). No child had a reported history of major medical, psychiatric, or learning disorder unrelated to PKU, and no child was treated with sapropterin dihydrochloride at the time of study.

Procedures

Approval to conduct this study was obtained from institutional review boards for the protection of human subjects at Washington University in St. Louis (WU) and Oregon Health & Science

University (OHSU). All participants and/or guardians provided written informed consent prior to initiation of study procedures. Referring metabolic clinics provided blood Phe levels over the lifetime based on medical records. Executive and neuroimaging procedures were administered during a single session lasting approximately 4 hours. Some data reported here were used in previous reports (e.g., Hood et al., 2014a, 2014b) but not in relation to mediation modeling.

Index of Phe Control

Mean Phe exposure over the lifetime was computed based on all available Phe levels prior to evaluation. The number of lifetime blood Phe levels for individual children who participated in our study ranged from 86 – 466 (M = 215.0, SD = 98.0). The rationale for examining this index is detailed elsewhere (Hood et al., 2014). Briefly, mean Phe exposure was computed to take into account the duration (i.e., years) and accumulative effects of exposure to elevations in Phe, because older children with PKU have experienced more prolonged exposure to elevated Phe than younger children. This method of calculation for mean exposure (M = 0, SD = 1.8, range = -2.5 – 4.5) results in scores that approximate a normal distribution, with higher scores indicating greater exposure.

Executive Abilities

The Matrix Reasoning subtest from the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) was used to measure nonverbal strategic processing. During this task, children viewed series of incomplete matrices and selected which of five solutions best completed each matrix. The number of correct completions was recorded. A verbal wordlist learning task was used to measure verbal strategic processing. Children listened as a list of 18 words was read aloud then orally recalled as many words as possible in any order over five learning trials. The list comprised six words from each of three semantic categories (e.g., furniture, food, parts of the

body). A ratio reflecting the number of words reported serially in semantic clusters to the total number of words recalled over the five trials was recorded. An n-back task with two conditions, location and letter, was used to measure working memory. Children observed 1 of 8 letters (C, F, H, J, N, P, Q, S) appearing alone at 1 of 8 locations along an imaginary circle on a computer monitor. In the location condition, children pressed a button when any letter appeared in the same location as two trials previously; in the letter condition, children pressed a button when the letter presented was identical to the letter presented two trials earlier. Otherwise, children withheld responses. The mean number of correct nonresponses averaged across location and letter conditions was recorded.

Standard scores (M = 100, SD = 15) from each task were used in mediation analyses. Matrix Reasoning standard scores in children with PKU (M = 99.3, SD = 11.1, range = 74.5 – 116.5) were based on age-referenced normative data that accompanied the subtest. Normative data were not available for the experimental wordlist learning and n-back tasks. However, to provide a similar interpretative context, we computed age-referenced standard scores for each task for children with PKU (wordlist learning: M = 99.4, SD = 8.2, range = 86.2 – 116.5; n-back task: M = 100.6, SD = 7.2, range = 89.0 – 113.1) based on data previously collected in our laboratory from a group of 80 healthy control children who ranged in age from 7 to 18 years (M = 12.4, SD = 3.2).

White Matter Integrity

Children were scanned with a 3.0T Siemens Trio at OHSU and with a 1.5T Siemens Sonata at WU. DTI was acquired using an echo planar imaging (EPI) sequence (TR = 9000 ms, TE = 84 ms (OHSU) and 78 ms (WU), 2.5 mm (OHSU) and 3.0 mm (WU) isotropic voxels, conventional hexahedral (6 direction) encoding with diffusion sensitization of b-values = 0 and 1000 s/mm2).

Four complete DTI datasets were acquired for each participant, with a total imaging time of approximately 1 hour. The first image-processing step was registration of all images. We defined the spatial relationships between all images in terms of affine transforms. T2W image registration was accomplished using vector gradient measure (VGM) maximization. The first acquired, unsensitized (b = 0 s/mm2; I0) DTI volume was registered to the T2W image; stretch and shear were enabled (12 parameter affine transform) to partially compensate for EPI distortion. The remaining DTI images were then registered to the unsensitized DTI volume. The diffusion tensor and its three eigenvalues were calculated using log-linear regression in each voxel for each ROI (Shimony 1999). Using standard methods, the DTI parameters were computed from the eigenvalues.

To minimize false-positives, we focused on two white matter brain regions: posterior parietal–occipital (PPO) and centrum semiovale (CSO) (see Figure 1). These regions were selected based on a well-established DTI atlas (Oishi et al., 2008), and placement of ROIs was compared on each participant's FA map and TW2 images simultaneously. ROIs were shifted by a few voxels as necessary by a trained neuroradiology technician to better conform to each individual's native anatomy. Finalized ROIs were then applied to each subject's mean diffusivity, axial diffusivity, and radial diffusivity parametric maps and sampled using Analyze 8.0 software similar to the methods of Shimony et al., 1999. Raters have established interrater correlation coefficients above 0.90 for mean diffusivity values for all ROIs.

We have previously shown that MD in these ROIs was significantly lower in individuals with PKU than in age-matched controls (White et al., 2013) and that MD in these ROIs was related to a range of indices of Phe control (Hood et al., 2014). In addition, visually observable white matter abnormalities often occur in the PPO and CSO in individuals with PKU (Citton,

2012). For mediation analyses, standard scores were generated for MD based on data from a subset (N = 62; not all children completed neuroimaging) of the healthy control children whose data were used to generate executive abilities standard scores.

Data Analyses

As a starting point in our analyses, we conducted Pearson correlations to determine the simple bivariate relationships between mean Phe exposure over the lifetime, MD in the PPO and CSO, and executive abilities. Statistical rigor was increased by considering findings significant only if $p < .05$ and effect sizes were either medium or large (Cohen, 1988).

For mediation analyses, we used a bootstrapping approach, which is a non-parametric resampling procedure for the assessment of indirect effects (Preacher & Hayes, 2004, 2008). Mediation analyses indicate whether the total effect (weight *c*) of an independent variable (IV; mean Phe exposure) on a dependent variable (DV; nonverbal strategic processing, verbal strategic processing, or working memory) comprises a direct effect (weight *c*') of an IV on a DV and an indirect effect (weight $a \times b$) of an IV on a DV through a predicted mediator (MD). Weight *a* denotes the effect of an IV on a mediator, whereas weight *b* denotes the effect of a mediator on a DV.

Current recommendations indicate that inferences should not be based on the significance of paths *a* and *b*; instead, inferences should be an explicit quantification of the indirect effect, and significant indirect effects can occur in the absence of significant total or direct effects (Preacher & Hayes, 2004, 2008). Given our small sample size, we focused on Kappa-squared (κ^2) to measure effect size because it is standardized and insensitive to sample size. In addition, due to small sample size and the exploratory nature of the current study, there was no correction for multiple statistical comparisons. To increase statistical rigor, indirect effects were considered

significant only if zero was not within the 95% confidence interval (CI), $p < .05$ two tailed, and effect size was medium or large. Given that three aspects of executive abilities and two ROIs were examined, a total of six mediation analyses were conducted.

We would also note that exploratory mediation analyses were conducted to determine whether subcomponents of MD, including axial diffusivity (AD) and radial diffusivity (RD), mediated the relationship between Phe exposure and executive abilities. Because results from these analyses were not explanatory beyond those conducted using MD, they are not discussed further.

Results

Correlation Analyses

Pearson correlations indicated that MD in the PPO was significantly related to nonverbal strategic processing and working memory, whereas MD in the CSO was significantly related to nonverbal and verbal strategic processing. MD in both the PPO and CSO was significantly related to mean Phe exposure. Executive abilities were not significantly related to mean Phe exposure, but because significant indirect effects may be present in the absence of total or direct relationships, we next conducted mediation analyses (Preacher & Hayes, 2004; Rucker et al., 2011).

Mediation Analyses

Of the six mediation analyses conducted, four yielded the statistically significant results of interest that are discussed here. As shown in figure 1, neither the total (weight *c*) nor direct (weight *c'*) effects of mean Phe exposure on nonverbal strategic processing were significant in analyses including either PPO or CSO as mediators (panels a and b, respectively).

Of greater interest, the indirect effects of mean Phe exposure on nonverbal strategic processing through both the PPO (panel a; 95% CI entirely below zero, $\kappa^2 = .24$, medium effect) and CSO (panel b; 95% CI entirely below zero, $\kappa^2 = .14$, medium effect) were statistically significant. In terms of verbal strategic processing (panel c), with CSO as the mediator, the total effect (weight *c*) of mean Phe exposure on verbal strategic processing was not significant, although the direct effect (weight *c'*) was significant. More importantly, the indirect effect of mean Phe exposure on verbal strategic processing through the CSO was statistically significant (95% CI entirely above zero, $\kappa^2 = .13$, medium effect). Turning to working memory (panel d), with PPO as the mediator, neither the total (weight *c*) nor direct (weight *c'*) effects of mean Phe exposure on working memory were significant. The indirect effect of mean Phe exposure on working memory through PPO, however, was statistically significant (95% CI below zero, $\kappa^2 = .26$, large effect).

Discussion

White matter compromise in individuals with PKU has been related to poorer Phe control (Anderson et al., 2007; Das et al., 2013; Hood et al., 2014), and this relationship has been shown more consistently when examined across the lifetime rather than at discrete points (Viau et al., 2011). More rarely, significant relationships have been shown between white matter integrity and cognition in individuals with PKU (Peng et al., 2004; Antenor-Dorsey et al., 2013). This is somewhat surprising, as compromised white matter integrity has been related to poorer cognition in many other neurological disorders (Chiaravalloti & DeLuca, 2008).

There are a number of possible reasons why direct relationships between white matter integrity and cognition have rarely been found in studies of PKU. First, cognition has most often been examined in relation to observable white matter abnormalities using structural MRI, which makes it difficult to identify subtle relationships (Pietz et al., 1996; Weglage et al., 2013). In

contrast, DTI permits detection of subtle disruptions in microstructural white matter integrity that are not detectable on structural images. Second, in previous studies, cognition has often been investigated using only IQ (Rupp et al., 2001). Such global measures, although useful, are less specific and do not address relationships with executive abilities, which are often impaired in individuals with PKU (Anderson et al., 2007; Christ et al., 2006). Finally, other studies have assessed relationships between cognition and white matter compromise in adults rather than children. It is possible that white matter compromise particularly affects cognition during earlier development.

In our exploratory study, mediation was used to assess the indirect effects of microstructural white matter integrity in PPO and CSO brain regions on the relationship between Phe exposure over the lifetime and executive abilities. Although both Phe and MD have been related to cognitive outcomes, such direct effects are not necessarily predictive of indirect effects. In fact, in our study, only one of four significant mediation models showed a significant direct effect of Phe on cognition. Overall, results from our study are the first to show that white matter integrity mediates the relationship between Phe and executive abilities and suggest that white matter integrity may be a sensitive marker of cognitive dysfunction. Our results also suggest that to fully understand the complex interplay between metabolic control, white matter integrity, and executive abilities in children with PKU, all of these domains should be analyzed in conjunction.

In addition to the strengths of our study, such as the use of mediation and DTI, there are limitations that should be acknowledged. For example, our exploratory study had a small sample size, which could have resulted in decreased power to detect additional significant effects, and so requires replication in a larger sample. In addition, because the study was cross-sectional,

causality was implied rather than determined. Future longitudinal research will be helpful in determining whether white matter integrity in childhood predicts executive abilities later in life and whether improvements in white matter integrity and in turn executive abilities can be obtained through lower lifetime Phe exposures.

Despite these limitations, our study provides unique information about neural processes that may affect cognition in children with PKU. Mediation analyses are rare in PKU research and have not previously been conducted in relation to executive abilities. We suggest that these types are analyses are crucial if we are to understand the complex relationships between metabolic control, neural factors, and functional outcomes in individuals with PKU.

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Table 1. Correlations between MD, Phe exposure, and executive abilities.

Variable	PPO	CSO
Nonverbal strategic processing	.34*	.35*
Verbal strategic processing	.22	.41*
Working memory	.36*	.19
Mean Phe exposure	-.65*	-.41*

Note: * = $p < .05$ with medium or large effect sizes.

Figure

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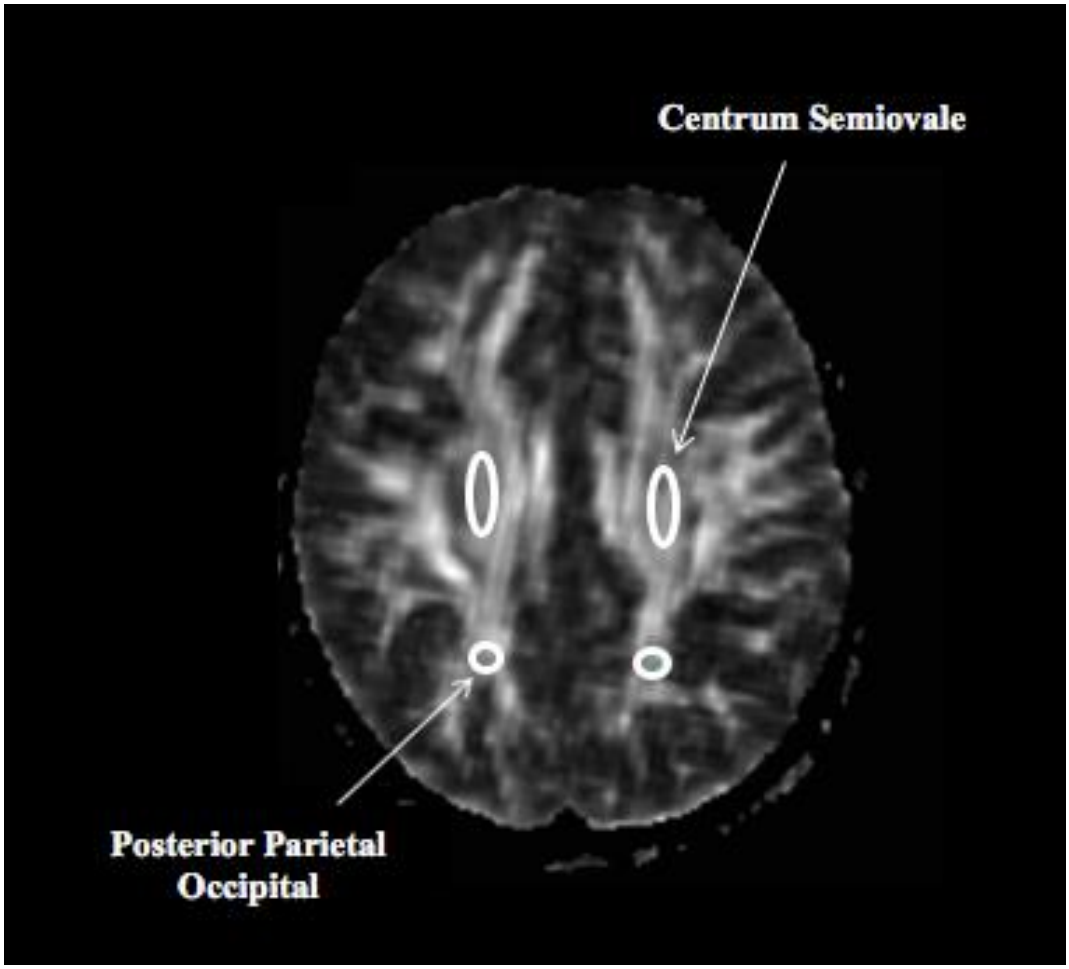

Figure 1. ROI placement

Figure 1. PPO and CSO as mediators of the relationship between mean Phe exposure and executive abilities