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White matter integrity and executive abilities in individuals with phenylketonuria

Jo Ann V. Antenor-Dorsey [a], Tamara Hershey [a,b,c], Jerrel Rutlin [a], Joshua S. Shimony [b], Robert C. McKinstry [b,d], Dorothy K. Grange [d], Shawn E. Christ [e], Desirée A. White [f,*]

[a] Department of Psychiatry, Campus Box 8134, Washington University School of Medicine, St. Louis, MO 63110, USA
[b] Mallinckrodt Institute of Radiology, Campus Box 8131, Washington University School of Medicine, St. Louis, MO 63110, USA
[c] Department of Neurology, Campus Box 8111, Washington University School of Medicine, St. Louis, MO 63110, USA
[d] Department of Pediatrics, Campus Box 8116, Washington University School of Medicine, St. Louis, MO 63110, USA
[e] Department of Psychological Sciences, 210 McAlester Hall, University of Missouri, Columbia, MO 65211, USA
[f] Department of Psychology, Campus Box 1125, Washington University, St. Louis, MO 63130, USA

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ABSTRACT

Previous studies have revealed white matter abnormalities in the brains of individuals with phenylketonuria (PKU), but the microstructural nature of these abnormalities and their relationship to phenylalanine (Phe) levels and cognitive outcomes are poorly understood. In the current study, the microstructural integrity of white matter in 29 individuals with early-treated PKU and 12 healthy controls was examined using two complementary diffusion tensor imaging (DTI) approaches: region-of-interest (ROI) based analysis and voxel-wise tract based spatial statistics (TBSS) analysis. Relationships among DTI, executive abilities, and Phe level findings were explored. DTI revealed widespread lowering of mean diffusivity (MD) in the white matter of the PKU group in comparison with the control group. Executive abilities were also poorer for individuals with PKU than controls. Within the PKU group, lower MD was associated with higher Phe level and poorer executive abilities. These findings are the first to demonstrate the interplay among microstructural white matter integrity, executive abilities, and Phe control in individuals with PKU.

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder in which metabolism of the amino acid phenylalanine (Phe) is disrupted due to phenylalanine hydroxylase deficiency; as a result, blood Phe levels are elevated in individuals with PKU [1]. The profound sequelae of untreated PKU, such as intellectual disability [1,2] and neurological abnormalities [3], are rarely seen in developed nations today due to newborn screening programs and early implementation of dietary treatment to limit Phe intake. Nonetheless, early-treated PKU is associated with lower than expected intelligence [4] and impairments in specific aspects of cognition, executive abilities in particular [5,6].

For decades it has been hypothesized that the cognitive sequelae of early-treated PKU are related to deficiencies in dopamine, a neurotransmitter of crucial importance in the function of frontal brain regions that subserve executive abilities [7]. There are two key mechanisms underlying dopamine deficiency in PKU. First, the neurochemical cascade by which Phe is converted to tyrosine and tyrosine is converted to dopamine (and other neurotransmitters) is disrupted because Phe is not properly metabolized. Second, available tyrosine must compete with excess Phe for passage across the blood–brain barrier via the large neutral amino acid type 1 (LAT1)-transporter, to which Phe binds more strongly than tyrosine [1].

Of particular relevance to the current investigation, in recent years investigators have turned their attention to white matter pathology as another neural mechanism by which cognition may be compromised. A number of studies have identified observable white matter abnormalities in individuals with PKU [8–17]. These abnormalities are generally characterized by hyperintensities in periventricular brain regions on T2-weighted images and are more pronounced at higher Phe levels. Although these studies based on qualitative examination of the white matter are informative, they are limited in terms of defining the nature of white matter pathology.

An advanced MRI approach, diffusion tensor imaging (DTI), holds promise for enhancing our understanding of white matter pathology. DTI provides detailed information regarding the microstructural integrity of white matter through the measurement of water molecule movement. This imaging approach is sensitive to subtle changes in white matter integrity [18], detecting pathology even when T2-weighted images appear normal [19]. Two DTI measures are most commonly reported: (1) mean diffusivity (MD), which indicates the overall spatial average rate of water molecule movement and (2) fractional anisotropy (FA), which indicates the degree of asymmetry of water molecule movement.

Reductions in MD but normal FA in white matter tracts have been reported in the studies of individuals with PKU, and more

* Corresponding author. Fax: +1 314 935 7588.
E-mail address: dawhite@wustl.edu (D.A. White).

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pronounced decreases in MD have generally been associated with higher blood Phe levels [10–13,15,17,20,21]. These findings indicate that the rate of water molecule movement is restricted in individuals with PKU compared with healthy controls. Most of the studies conducted to date, however, have suffered from limitations such as small sample size or inclusion of both early- and later-treated individuals within the same study. In some studies, DTI was examined only in brain regions having observable white matter abnormalities on T2-weighted images, which may have resulted in failure to identify important changes in other brain regions. Moreover, although changes in MD have been examined in relation to Phe levels, they have not been examined in relation to executive abilities.

In the present study, we conducted a comprehensive examination of the microstructural integrity of the white matter across the brain in a relatively large number of individuals with early-treated PKU. To do so, two complementary DTI approaches were used: (1) region of interest (ROI) analysis and (2) voxel-wise tract based spatial statistics (TBSS) [22] analysis. In ROI analysis, the brain regions to be examined are established a priori. ROI analysis has several advantages over the voxel-wise analysis, including minimization of type 1 error, minimization of statistical assumptions, and tailoring of ROIs to avoid partial volume effects which reduce the impact of imperfect registration procedures [18,23]. On the other hand, voxel-wise analysis considers all voxels within all major white matter tracts without regard to arbitrary or unreliable boundaries and without a priori assumptions. We employed both approaches to provide a comprehensive overview of the white matter microstructure of individuals with PKU. In addition, Phe level, IQ, and executive abilities were examined in relation to DTI findings. This study is the first to elucidate interrelationships between microstructural white matter integrity, metabolic control, and executive abilities.

2. Material and methods

2.1. Participants

Individuals with PKU (n = 32; 12 females, 20 males) were recruited through metabolic clinics at Washington University (WU; n = 13), University of Missouri (UM; n = 9), University of Florida (n = 4), St. Louis University (n = 3), New York Medical College (n = 2), and University of Nebraska (n = 1). Preliminary analyses revealed no significant differences in cognitive or neuroimaging findings between the two sites (WU and UM) from which the majority of participants with PKU were recruited ($p > .05$ in all instances). All individuals with PKU were diagnosed soon after birth and were treated early through dietary management to limit Phe intake. Blood Phe obtained closest to the time of cognitive and neuroimaging evaluations (typically the same day) ranged from 115 to 1459 μmol/L (M = 734, SD = 410), which is elevated in comparison with blood Phe in healthy individuals without PKU (i.e., ≤120 μmol/L).

Findings from individuals with PKU were compared with those of healthy controls (n = 12; 4 females; 8 males) recruited from the St. Louis community. No participant had a reported history of major medical (e.g., stroke), psychiatric (e.g., depression), or learning (e.g., dyslexia) disorder unrelated to PKU. Age ranged from 6 to 35 years (M = 18.0, SD = 9.0) for the PKU group and 7 to 33 years (M = 17.8, SD = 8.0) for the control group. Education ranged from 0 to 18 years (M = 9.1, SD = 4.6) for the PKU group and 1 to 16 years (M = 10.3, SD = 4.8) for the control group. With regard to race/ethnicity, 3% and 8% of the PKU and control groups, respectively, comprised individuals from minority populations. There were no significant between-group differences in age, education, or race/ethnicity ($p > .05$ in all instances).

2.2. Procedures

Data from this report are components of a larger study examining the effects of sapropterin dihydrochloride on brain and cognition in individuals with early-treated PKU. Approval to conduct this study was obtained from institutional review boards for the protection of human subjects at WU and UM, the institutions at which neuroimaging and cognitive data for the study were collected. Written informed consent was obtained for all participants and/or their guardians prior to engagement in study procedures. Participants typically completed neuroimaging and cognitive evaluations on the same day in a session lasting approximately 4 h. A manuscript involving voxel-wise analyses that included data from a small subset of participants in the current study (n = 9) is under review elsewhere, but neither ROI analyses nor cognitive findings were included in that study.

2.3. Neuroimaging

Structural images were acquired on a Siemens TIM Trio 3.0 T imaging system (Erlangen, Germany) with a standard Siemens 12 channel head coil. These images included a T1-weighted (T1W) sagittal, magnetization-prepared rapid gradient echo [MPRAGE; repetition time (TR) = 2000 ms (WU and UM), echo time (TE) = 3.03 ms (WU) and 2.97 (UM), flip angle = 8° (WU and UM), FOV = 256 × 256 pixels (WU) and 256 × 224 (UM), voxel resolution = 0.88 × 0.88 × 0.9 mm (WU and UM) and a T2-weighted (T2W) fast spin echo [TR = 3200 (WU and UM), TE = 475 (WU and UM), flip angle = 120° (WU and UM), FOV = 256 × 256 pixels (WU and UM), voxel resolution = 0.88 × 0.88 × 0.9 mm (WU and UM)].

DTI was acquired using an echo planar imaging (EPI) sequence [TR = 12,437 (WU) and 9900 (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 (DWI) with variable b factor up to 1000 s/mm^2 maximum were acquired along 25 non-collinear diffusion gradient orientations. DWIs were registered first to the b = 0 unsensitized image, then to the T2W, then to the best T1W (MPRAGE), and finally to an in-house atlas constructed at WU. Parametric maps were then generated for MD and FA.

MD and FA analyses were conducted using two complementary approaches: (1) ROI analysis and (2) voxel-wise analysis using TBSS [24]. ROIs were selected based on a well-established DTI atlas [25] and verified by a neuroradiologist. They were then applied to each participant's MD and FA parametric maps and sampled using Analyze version 8.0 (Mayo Clinic, Rochester). We focused on the following 10 ROIs to provide a sampling across various brain regions: prefrontal cortex, centrum semiovale, posterior parietal-occipital cortex, optic radiation, putamen, corpus callosum (genu, body, splenium), thalamus, and hippocampus. Values from left and right homologous regions were averaged.

As a complementary approach, we used voxel-wise analysis to confirm ROI findings and to permit identification of PKU-related white matter findings in other regions without regard for strict anatomical boundaries. Using each individual's motion-corrected, aligned, and averaged DWI dataset, BET (FMRIB Brain Extraction Tool) was used to compute a brain mask, and FDT (FMRIB diffusion toolbox) was used to compute FA and MD images. These images were projected onto a skeleton and set at a threshold of FA = 0.2 for voxel-wise analysis.

2.4. Cognition

General intellectual ability (IQ) and executive abilities (working memory, strategic processing) were assessed for all participants.

2.4.1. General intellectual ability

IQ was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI), which comprises four subtests (i.e., Vocabulary, Similarities, Block Design, Matrix Reasoning). A composite score