Molecular Genetics and Metabolism xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Prolonged exposure to high and variable phenylalanine levels over the lifetime predicts brain white matter integrity in children with phenylketonuria

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ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form 4 November 2014
Accepted 6 November 2014
Available online xxxx

Keywords:
Phenylketonuria
Phenylalanine
Exposure
Brain
White matter
Diffusion tensor imaging

ABSTRACT

In this study, we retrospectively examined the microstructural white matter integrity of children with early- and continuously-treated PKU ($N = 36$) in relation to multiple indices of phenylalanine (Phe) control over the lifetime. White matter integrity was assessed using mean diffusivity (MD) from diffusion tensor imaging (DTI). Eight lifetime indices of Phe control were computed to reflect average Phe (mean, index of dietary control), variability in Phe (standard deviation, standard error of estimate, % spikes), change in Phe with age (slope), and prolonged exposure to Phe (mean exposure, standard deviation exposure). Of these indices, mean Phe, mean exposure, and standard deviation exposure were the most powerful predictors of widespread microstructural white matter integrity compromise. Findings from the two previously unexamined exposure indices reflected the accumulative effects of elevations and variability in Phe. Given that prolonged exposure to elevated and variable Phe was particularly detrimental to white matter integrity, Phe should be carefully monitored and controlled throughout childhood, without liberalization of Phe control as children with PKU age.

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder characterized by disruption in the metabolism of the amino acid phenylalanine (Phe) due to a deficiency in or an absence of the phenylalanine hydroxylase (PAH) enzyme; as a result, Phe levels are elevated in individuals with PKU [1]. If untreated, PKU typically results in significant neurologic compromise and intellectual disability [2]. These severe sequelae are avoided through early detection and dietary restrictions to limit Phe intake. Nonetheless, individuals with early- and continuously-treated PKU are at risk for compromised functional outcomes such as neuropsychological impairments [3,4] psychosocial difficulties [5,6], and psychiatric disorders [7].

The brain mechanisms underlying diminution of functional outcomes are not fully understood. Previous research has largely focused on disruptions in dopamine synthesis [8,9]. However, there are also reports of increased oxidative stress [10], disrupted protein synthesis [11], and disrupted glucose metabolism [12] in individuals with PKU.

Of particular relevance to the current research, neuroimaging studies have revealed that PKU is associated with widespread compromise of the white matter of the brain. Most research has focused on white matter abnormalities that were detectable via visual inspection of structural brain images [9,13–20]. Categorical labels were then typically assigned to indicate the severity of the identified abnormalities. Although useful, this qualitative approach provides little information regarding subtle compromise in the white matter that is not obvious via visual inspection.

Recently, diffusion tensor imaging (DTI) has been used to examine the microstructural white matter integrity of individuals with PKU. This refined neuroimaging approach is sensitive to subtle white matter compromise and provides quantitative data that offer insight into the nature of white matter pathology. DTI studies have consistently shown that mean diffusivity (MD) is significantly lower across a range

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http://dx.doi.org/10.1016/j.ymgme.2014.11.007
1096-7192/

of brain regions in individuals with PKU in comparison with healthy controls [21–27], suggesting that the average diffusivity of water along all directions is restricted.

In terms of relationships with Phe, a number of studies have shown that greater white matter compromise (whether visible abnormalities or lower MD) is associated with higher Phe at the time of neuroimaging (i.e., concurrent Phe) or average Phe over a period of time preceding neuroimaging. It could be equally or more informative to investigate other indices of Phe control in relation to white matter compromise. However, in the only study to do so, the severity of visible white matter abnormalities was not associated with variability in Phe as measured by the standard error of estimate of Phe in relation to age (i.e., index of dietary control fluctuation) [18].

Although the studies described thus far are clearly of interest, many have been limited by the qualitative approach used to define white matter compromise. With few exceptions, white matter compromise was examined in relation to a single measurement of Phe or a single index of average Phe that was calculated over a brief period rather than over the lifetime. Some studies also suffered from small sample size or included a mixed group of individuals with PKU who were and were not on dietary Phe restriction. Finally, to our knowledge, no studies have been conducted using DTI to examine white matter integrity in relation to indices of Phe control other than concurrent Phe or average Phe over a period of time.

To address the aforementioned issues, the current study examined eight indices of Phe control over the lifetime to determine which best predicted microstructural white matter integrity in a relatively large sample of school-age children with early- and continuously-treated PKU. Two indices reflected average Phe, three indices reflected variability in Phe, and one index reflected change in Phe with age. In addition, two previously unstudied indices reflected accumulative exposure to Phe over the lifetime.

2. Material and methods

2.1. Participants

Children with PKU ($n = 36$; 17 male, 19 female) were recruited through metabolic clinics at Washington University in St. Louis ($n = 11$), Oregon Health & Science University ($n = 19$), the University of Missouri ($n = 3$), New York Medical College ($n = 1$), the University of Florida ($n = 1$), and the University of Nebraska ($n = 1$). All children were diagnosed with PKU soon after birth and received early treatment through dietary management to limit Phe intake. Lifetime Phe levels, with gaps of no more than 2 years prior to neuroimaging, were retrospectively available for all children. At the time of neuroimaging, 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.

2.2. Procedures

We obtained approval to conduct this study from institutional review boards for the protection of human subjects at Washington University in St. Louis, Oregon Health & Science University, and the University of Missouri, which were the sites at which DTI data were collected. All participants and/or their guardians provided written informed consent prior to engagement in study procedures. Neuroimaging data were collected as components of larger studies that included measures of cognition. Administration of all cognitive and neuroimaging procedures occurred during a single session lasting approximately 4 h. The metabolic clinics from which children were referred provided blood Phe levels over the lifetime based on available medical records. Some of the Phe and neuroimaging data reported here have been published previously [21,28], but not in terms of relationships between various indices of Phe control and DTI findings.

2.3. Measures

2.3.1. Indices of Phe control

With the exception of mean exposure and SD exposure, the indices of blood Phe control are described in detail elsewhere [28]. Briefly, we computed 8 indices over the lifetime. Average Phe was represented by mean Phe and the IDC; mean Phe was the mean of all available Phe levels, whereas the IDC was the mean of all yearly median Phe levels. Variability in Phe was reflected by the SD Phe, SEE Phe, and % spikes; SD Phe was the degree of variation in Phe around the mean; SEE Phe was the degree of variation in Phe around a regression function; and % spikes was the proportion of Phe levels that were at least 600 μmol/L greater than either the preceding or succeeding Phe level in relation to the total number of Phe levels available. Change in Phe with age was represented by the slope of a regression function in which Phe was plotted in relation to age.

Finally, two previously unexamined indices of Phe control, mean exposure and SD exposure, were computed to take into account the duration (i.e., years) and accumulative effects of exposure to elevations and variability in Phe. The rationale for examining these two indices was that older children with PKU have experienced more prolonged exposure to elevations and variability in Phe than younger children. The approach used to compute the exposure indices was comparable to that used by Perantie et al. 2007 [29] to examine exposure to hyperglycemia and hypoglycemia in children with diabetes. As the first step in calculating mean exposure, we obtained the mean and standard deviation for both mean Phe and age across the entire sample of children with PKU. Z scores for mean Phe and age were then computed for each child based on the means and standard deviations of the sample. Mean exposure for each child was then calculated by summing the *z* scores [30] for mean Phe and age. SD exposure was similarly computed based on the mean and standard deviation for SD Phe and age across the entire sample of children with PKU. This method of calculation results in scores that approximate a normal distribution, with higher scores indicating greater exposure to Phe or variability in Phe.

2.3.2. Diffusion tensor imaging

Neuroimaging procedures are described in detail elsewhere [21]. Briefly, DTI data were acquired using a diffusion weighted echo planar imaging sequence along 6 (24 children) and 25 (12 children) non-collinear diffusion gradients (maximal b value of 1000 s/mm^2 and voxel size 2.0 mm^3) on a Siemens Tim Trio 3.0 T imaging system (Erlangen, Germany). Diffusion weighted images were registered to an in-house atlas at Washington University, and parametric maps were generated for MD. Fractional anisotropy was not examined, because previous studies have consistently found few differences between individuals with PKU and healthy controls on this DTI variable [21,22,24–27].

MD was examined using two approaches: region of interest (ROI) analyses and tract based spatial statistics (TBSS) analyses. ROI analyses focused on 10 brain regions: prefrontal cortex, centrum semiovale, posterior parietal–occipital cortex, optic radiation, hippocampus, corpus callosum (genu, body, splenium), thalamus, and putamen; data for left and right homologous brain regions were averaged. TBSS analyses were used to examine microstructural white matter integrity without regard for strict anatomical boundaries.

2.3.3. Data analyses

For ROI analyses, *z* scores for MD in the 10 noted brain regions were generated for each child with PKU based on ROI data (i.e., mean and SD) previously collected in our laboratory from 62 typically-developing healthy children with an age range comparable to that of our PKU sample (details regarding the healthy sample are reported elsewhere [26]).

Please cite this article as: A. Hood, et al., Prolonged exposure to high and variable phenylalanine levels over the lifetime predicts brain white matter integrity in children with phenylketonuria, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.11.007