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Morphometric analysis of gray matter integrity in individuals with early-treated phenylketonuria

Shawn E. Christ [a,*], Mason H. Price [a], Kimberly E. Bodner [a], Christopher Saville [a], Amanda J. Moffitt [b], Dawn Peck [c]

[a] Department of Psychological Sciences, University of Missouri, Columbia, MO, United States
[b] Department of Human Development and Family Studies, University of Missouri, Columbia, MO, United States
[c] Department of Child Health, University of Missouri, Columbia, MO, United States

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ABSTRACT

The most widely-reported neurologic finding in individuals with early-treated phenylketonuria (PKU) is abnormality in the white matter of the brain. In contrast, much less is known regarding the impact of PKU on cortical gray matter (GM) structures. Presently, we applied advanced morphometric methods to the analysis of high-resolution structural MRI images from a sample of 19 individuals with early-treated PKU and an age- and gender-matched comparison group of 22 healthy individuals without PKU. Data analysis revealed decreased GM volume in parietal cortex for the PKU group compared with the non-PKU group. A similar trend was observed for occipital GM volume. There was no evidence of group-related differences in frontal or temporal GM volume. Within the PKU group, we also found a significant relationship between blood phenylalanine levels and GM volume for select posterior cortical sub-regions. Taken together with previous research on white matter and gray matter abnormalities in PKU, the present findings point to the posterior cortices as the primary site of neurostructural changes related to early-treated PKU.

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive condition characterized by a deficiency in phenylalanine hydroxylase (PAH). PAH represents an essential enzyme for the metabolism of the amino acid phenylalanine (phe) into tyrosine. Within this context, PKU is associated with depletions in tyrosine and its metabolites (e.g., dopamine) as well as elevated levels of phe. Other large neutral amino acids (LNAAs; e.g., tryptophan) and their metabolites (e.g., serotonin) are also affected to the extent that excess phe competitively inhibits the transport of these LNAAs across the blood-brain barrier [1].

Even with early and continuous treatment, individuals with PKU experience cognitive and neurologic disruptions (albeit not as severe as that resulting from untreated PKU). Early-treated PKU (ETPKU) is associated with a slight decrease in overall IQ coupled with circumscribed impairment in a handful of domains including executive function, processing speed, and emotional regulation [2,3].

In terms of neurologic outcome in ETPKU, previous research has focused almost exclusively on the effects on white matter. Indeed, white matter abnormalities represent the most evident and widely-reported neurologic finding in individuals with ETPKU. Based on a review of the literature, Anderson and Leuzzi [4] estimated the prevalence of white matter abnormalities at more than 90% among individuals with ETPKU. The most common locus of such abnormalities is bilateral posterior aspects of the white matter surrounding the ventricles [5]. The extent and severity of the white matter abnormalities appear to be moderated by patient age and dietary adherence (as reflected by blood phe levels), with older age and higher phe levels associated with increased white matter involvement [6,7]. In more severe cases, the affected area extends to include anterior ventricular and subcortical white matter [8].

In contrast, very little is known regarding potential effects of ETPKU on gray matter structures in the brain. Early studies, which relied on visual evaluation of neuroimaging scans, reported no evidence of gray matter (GM) involvement [8,9]. More recent studies utilizing more quantitative methodology (e.g., volumetric analysis) have yielded mixed results. For example, a study by Pfaender et al. [10] found significantly smaller hippocampus, pons, and cerebrum volumes in a sample of individuals with ETPKU compared to a typically developing non-PKU sample. They found no group differences in basal ganglia, cerebellum, or thalamus volume. In contrast, Pérez-Dueñas et al. [11] reported volume reductions in thalamus as well as motor cortex and premotor cortex in a mixed sample of individuals with early-treated and late-treated PKU. They also found a significant PKU-related increase in GM volume within the ventral striatum. More recently, Bodner et al. [12] reported

Abbreviations: PKU, phenylketonuria; PAH, phenylalanine hydroxylase; Phe, phenylalanine; LNAAs, Large neutral amino acids; ETPKU, early-treated phenylketonuria; GM, gray matter; MRI, magnetic resonance imaging; CBA, cortex-based alignment; FSIQ, full scale intelligence quotient; VBM, voxel-based morphometry.

* Corresponding author at: Department of Psychological Sciences, 210 McAlester Hall, University of Missouri, Columbia, MO 65203, United States.
E-mail address: christse@missouri.edu (S.E. Christ).

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increased putamen volume in a sample of individuals with ETPKU compared to individuals without ETPKU.

The present study sought to further examine potential ETPKU-related abnormalities in cortical GM thickness and volume. To this end, we applied recent and refined methodological advances in neuroimaging to the analysis of high-resolution structural MRI images from a sample of 19 individuals with ETPKU and a comparison group of 22 healthy individuals without PKU.

2. Methods

2.1. Participants

A sample of 19 individuals with ETPKU (9 males, 10 females) ranging in age from 9 to 33 years ($M = 21.4, SD = 8.0$) participated in this study. Diagnosis of PKU was made and treatment was implemented shortly after birth, as indicated by medical records or parental report. Unfortunately, complete lifetime records of blood phe level could not be attained for several older participants in the sample. Recent blood phe levels, however, were available for all participants. Although all patients reported that they had continued to maintain phe-restricted dietary treatment, a wide range of blood phe levels was observed in the PKU group. Mean phe levels in the year prior study enrollment ranged from 161 to 1459 μmol/L (group $M = 747$; $SD = 335.0$). Individuals with severe cognitive impairment or major medical disorders unrelated to PKU were excluded.

A comparison sample of 22 neurologically uncompromised individuals (11 males, 11 females) ranging in age from 9 to 33 years ($M = 20.7, SD = 8.1$) also participated in this research. Healthy non-PKU participants were recruited from the Columbia, Missouri community via advertisements/postings and word-of-mouth. Prior to enrollment, the potential participants were asked to complete an extensive questionnaire detailing past developmental and medical history. Individuals with significant medical and/or psychiatric history were excluded. The ETPKU and control groups did not differ in either age or gender ($p > 0.75$ in both instances).

The Wechsler Abbreviated Scale of Intelligence (Psychological Corporation 1999) was administered to estimate general intellectual ability. For individuals in the ETPKU group, scores ranged from 73 to 124, with a mean of 101.0 ($SD = 13.4$). For individuals in the control group, scores ranged from 93 to 133, with a mean of 112.4 ($SD = 10.1$). The scores of the control group were significantly higher than those of the ETPKU group [$t(39) = 3.10$, $p = 0.004$, $d = 0.99$].

2.2. MRI data acquisition

The present structural dataset was gathered in conjunction with our recent and ongoing functional MRI research on ETPKU [13,14]. The research protocol was approved by the Institutional Review Board of the University of Missouri, Columbia. Written informed assent and consent was obtained from all participants at the time of their visit.

Data for 25 participants (12 PKU, 13 non-PKU) were obtained on a 3 T Siemens Trio MRI scanner with a standard 8-channel head coil was used to obtain high-resolution (1 mm^3) $T-1$ weighted structural images of the brain. Images were collected using a standard T1-weighted pulse sequence [MP-RAGE sequence: TR = 2400 ms, TE = 3.16 ms, flip angle = 8°, in-plane resolution = 1 × 1 mm, slice thickness = 1 mm, number of slices = 176]. Data for the remaining 16 participants (7 PKU, 9 non-PKU) were obtained on a 1.5 T Siemens Symphony scanner with a standard 8-channel head coil and similar scan parameters [MP-RAGE sequence: TR = 1920 ms, TE = 3.75 ms, flip angle = 8°, in-plane resolution = 1 × 1 mm, slice thickness = 1 mm, number of slices = 160]. Note that, in instances where multiple scan images were available for a given participant, the images were averaged so as to provide a single high-quality image for further processing.

2.3. Data processing & analysis

Data processing and analysis was carried out using BrainVoyager QX software (Brain Innovation, Maastricht, The Netherlands). The standard BrainVoyager processing pipeline was used for cortical alignment and thickness analysis with a few noted exceptions: First, the structural MR image data for each participant was rotated into AC-PC coordinates. The skull and dural tissue were then be removed by manually deleting voxels containing non-neural tissue. Next, a Sigma filter and BrainVoyager's automatic intensity inhomogeneity correction tool were applied to enhance GM/WM tissue contrast and correct for spatial intensity inhomogeneities, respectively. Non-cortical structures (e.g., ventricles, subcortical nuclei) were then removed, and the resulting image was upsampled from 1 mm^3 to 0.5 mm^3 using sinc interpolation (the upsampling is required for use of BrainVoyager's advanced segmentation tools). BrainVoyager's built-in segmentation process, which uses local intensity histograms and computed gradient fields to adaptively calculate WM, GM, and CSF boundaries, was then applied. Importantly, before proceeding, the resulting segmentations were visually inspected (slice-by-slice) for accuracy and manually corrected where necessary. Such manual segmentation, while time consuming, is considered the "gold-standard" by most investigators for detecting subtle volumetric differences [15]. Lastly, the resulting segmentations were used to calculate cortical thickness measurements using a Laplace method [16] as implemented within BrainVoyager.

Output from the previously described segmentation process was also utilized to create cortical surface reconstructions of each hemisphere for each participant. Anatomical alignment of these surface representations (and thereby the aforementioned cortical thickness measurements) across participants was accomplished using BrainVoyager's automatic cortex-based alignment (CBA) process [17,18]. CBA represents an iterative adaptive process whereby curvature information (representing gyral and sulcal folding patterns) is used to align macro-anatomical structures (gyri and sulci) of each participant's brain to a standard reference brain provided in BrainVoyager. Following alignment, average gray matter volumetric measurements were extracted for each major cortical lobe (frontal, parietal, occipital, temporal) as well as individual sulcal and gyral subregions (e.g., inferior frontal gyrus, superior temporal sulcus, inferior parietal lobule) of each participant.

3. Results

A series of hierarchical regression analyses were conducted, with the volume for each cortical gray matter structure serving as the dependent variable in turn. In order to account for the potential contribution of age and gender to GM volume, these two factors were entered in the first step of the regression model. Group (PKU and non-PKU) was entered into the second step, followed by the two-way interaction variables (age × gender; group × age; group × gender) in the third step. The three-way interaction variable (age × gender × group) was entered into the fourth and final step of the model. To protect against an increase in the likelihood of a Type I error related to multiple comparisons, we confirmed a group effect on lobe volume (frontal, temporal, parietal, and/or occipital) before proceeding to analyze specific structures (gyri and sulci) located in the respective lobe.

Mean volumes for cortical GM structures for both groups are shown in Fig. 1. After accounting for variance related to age and gender, parietal GM volume was significantly smaller for the PKU group ($M = 117.5$ cm^3) compared to the non-PKU group ($M = 124.8$ cm^3) [$F(1,36) = 4.80$, $p = 0.04$, $pr^2 = 0.12$]. There was also a trend towards smaller occipital GM volume in the PKU group ($M = 47.5$ cm^3) compared to the non-PKU group ($M = 50.2$ cm^3) [$F(1,36) = 3.41$, $p = 0.07$, $pr^2 = 0.09$]. There was no evidence of group differences in either frontal lobe or temporal lobe GM volume [$F(1,36) < 0.20$, $p > 0.70$, $pr^2 < 0.01$ in both instances]. In addition, no significant group-related