



The effects of early-treated phenylketonuria on volumetric measures of the cerebellum

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ABSTRACT

Past murine studies of phenylketonuria (PKU) have documented significant effects on cerebellum at both the gross and cellular levels. The profile of neurocognitive and motor difficulties associated with early-treated PKU (ETPKU) is also consistent with potential cerebellar involvement. Previous neuroanatomical studies of cerebellum in patients with PKU, however, have yielded mixed results. The objective of the present study was to further examine potential differences in cerebellar morphometry between individuals with and without ETPKU. To this end, we analyzed high resolution T1-weighted MR images from a sample of 20 individuals with ETPKU and an age-matched comparison group of 20 healthy individuals without PKU. Measurements of whole brain volume, whole cerebellum volume, cerebellar gray matter volume, and cerebellar white matter volume were collected by means of semiautomatic volumetric analysis. Data analysis revealed no significant group differences in whole brain volume, whole cerebellar volume, or cerebellar white matter volume. A significant reduction in cerebellar gray matter volume, however, was observed for the ETPKU group compared to the non-PKU comparison group. These findings expand on previous animal work suggesting that cerebellar gray matter is impacted by PKU. It is also consistent with the hypothesis that the cognitive difficulties experienced by individuals with ETPKU may be related to disruptions in gray matter. Additional studies are needed to fully elucidate the timing and extent of the impact of ETPKU on cerebellum and the associated neurocognitive consequences.

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder affecting metabolism of the amino acid phenylalanine (Phe) into tyrosine, a precursor for dopamine and other neurotransmitters. The net results of altered Phe metabolism in PKU include decreased neurotransmitter production, oxidative stress, and disruption in protein synthesis [1–3]. The severity of these results can be ameliorated by early and continuous implementation of a Phe-restricted diet [4,5]. Although individuals with early-treated PKU (ETPKU) are spared the more severe impairments associated with untreated PKU [6], they do experience some neurologic and cognitive sequelae. ETPKU is associated with impairment of executive aspects of language, memory, learning, and attention control [7–10]. Additional work has also shown individuals with

ETPKU to have deficits in fine motor control and information processing speed [11–15].

Recent work has shown that deficits in learning, attention, language (including verbal working memory), as well as motor control and information processing are observed in individuals with damage to the cerebellum [16,17]. Although the cerebellum has traditionally been associated with motor skills and coordination via its projections to primary motor cortex, it is now known that it also has similar reciprocal connections with non-motor prefrontal cortex [18,19], a region that plays a critical role in higher order cognitive abilities such as executive function [20] and is known to be affected in ETPKU [21,22].

Little is known regarding the potential impact of ETPKU on the cerebellum. Atypical findings such as reduced cell count and dendritic arborization of cerebellar Purkinje cells are noted in a handful of early

Abbreviations: PKU, phenylketonuria; ETPKU, early-treated phenylketonuria; MRI, magnetic resonance imaging; Phe, phenylalanine; WBV, whole brain volume; WCV, whole cerebellum volume; GGM, cerebellar gray matter volume; CWM, cerebellar white matter volume; CSF, cerebrospinal fluid

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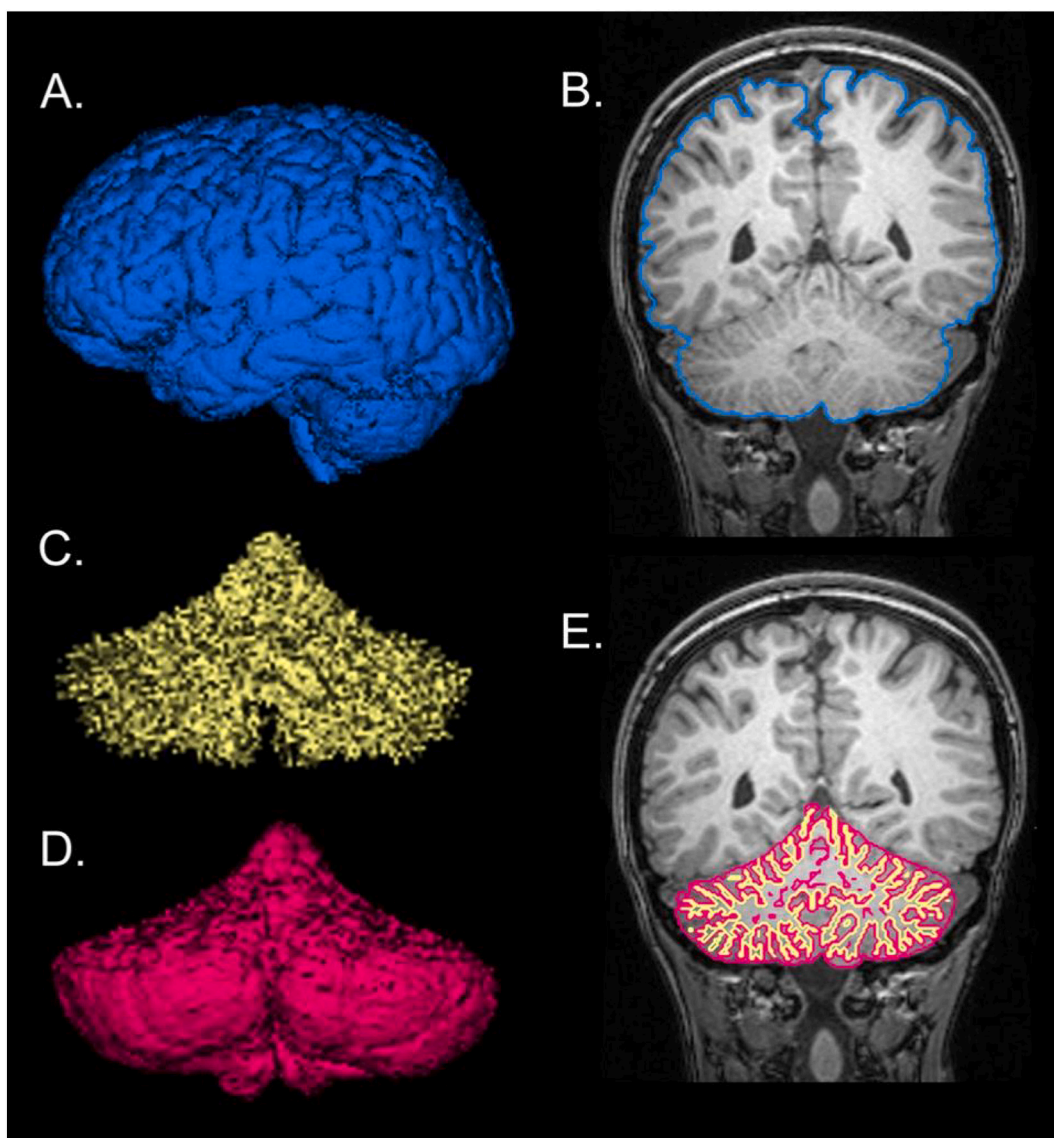


Fig. 1. Illustration of example volume segmentations using Analyze © software. A) 3D reconstruction of WBV; B) coronal view of segmentation of WBV; C,D) 3D reconstructions of cerebellar WM and GM, respectively; E) coronal view of segmentation of cerebellar WM and GM.

histological case studies of untreated PKU [23,24]. Findings for ETPKU are less clear. Whereas one study found depressed glucose metabolism in cerebellum in a sample of individuals with ETPKU [25], neuroimaging studies of gross anatomical morphology and structure have yielded little evidence of ETPKU-related cerebellar abnormalities. For example, a study by Hajek et al. [26] found lesions in cerebellar white matter in only a small portion (20%) of a sample of 20 individuals with ETPKU and Pietz et al. [27] found < 10% to have lesions in a sample of 51 subjects. In another study, Pearsen et al. reported no visual evidence of involvement of cerebellar gray or white matter in a sample of 15 patients with ETPKU [28]. Lastly, Pfaender et al. found no significant difference in overall cerebellar volume between individuals with and without ETPKU [29].

1.1. The present study

The goal of the present study was to evaluate the effects of ETPKU on the cerebellum by examining different aspects of cerebellar volume within a single sample of individuals with ETPKU. We used structural MRI to examine cerebellar volume, as well as volume of the gray matter (GM) and white matter (WM) components of the cerebellum separately.

Given the particular cognitive and motor difficulties observed in individuals with ETPKU and their association with the cerebellum and its connections (particularly with frontal cortex), we hypothesized that cerebellar dysmorphology would be present in individuals with ETPKU.

2. Methods

2.1. Participants

Our study sample consisted of 20 participants (11 males, 9 females) with ETPKU ranging in age from 11 to 27 years ($M = 16.0$, $SD = 3.8$). Individuals with ETPKU were diagnosed shortly after birth and a Phe-restricted dietary treatment was implemented at that time, as indicated by medical records and/or patient report. Complete lifetime records of blood Phe levels were available for 16 of the 20 of the participants in the sample ($M = 465.6 \mu\text{mol/L}$, $SD = 242$). Recent blood Phe levels were available for 19 of the 20 participants; the mean blood Phe level over the year prior to testing was $620.5 \mu\text{mol/L}$ ($SD = 369$), and the mean level over the three months prior to testing was $593.3 \mu\text{mol/L}$ ($SD = 356$). None of the participants were being treated with sapropterin at the time of testing. Individuals with major medical

Table 1

Structural volumes in non-PKU and ETPKU individuals. Whole brain volume (WBV), whole cerebellar volume (WCV), cerebellar gray matter volume (CGM), and cerebellar white matter volume (CWM). Volumes measured in mm³.

	Non-PKU n = 20		ETPKU n = 20		ETPKU/Non-PKU
	Volume	% WBV	Volume	% WBV	Mean
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	% difference
WBV	1,112,057 (147939)	–	1,172,660 (129897)	–	+ 5.4%
WCV	121,680 (15532)	10.99 (0.98)	122,218 (17886)	10.41 (0.90)	- 5.6%
CGM	77,285 (10664)	6.98 (0.67)	75,820 (12977)	6.45 (0.76)	- 8.1%
CWM	44,395 (6031)	4.02 (0.45)	46,398 (6799)	3.96 (0.42)	- 1.4%

disorders unrelated to PKU or severe cognitive impairment were excluded.

A sample of 20 healthy individuals without PKU (10 males, 10 females) ranging in age from 11 to 27 years ($M = 16.2$, $SD = 3.8$) comprised an age- and gender-matched control group. Healthy non-PKU participants were recruited from the Columbia and St. Louis, Missouri communities.

The Wechsler Abbreviated Scale of Intelligence [30] was administered to estimate general intellectual ability. For individuals in the ETPKU group, scores ranged from 76 to 124, with a mean of 106.3 ($SD = 11.8$). For individuals in the control group, scores ranged from 84 to 139, with a mean of 113.9 ($SD = 13.4$). The scores of the non-PKU group were higher than those of the ETPKU group though not statistically significantly different [$t(38) = 1.90$, $p = 0.07$].

2.2. MRI data acquisition

The present study was approved by the University of Missouri Internal Review Board (Review ID 1091119) and was carried out in accordance with the provisions of the World Medical Association Declaration of Helsinki. Informed consent was obtained for all individuals prior to participation.

High-resolution T1-weighted MP-RAGE magnetic resonance imaging (MRI) data were collected for all individuals with a 1.5 T scanner (TR = 1920 ms, TE = 4 ms, flip angle = 8°, in-plane

resolution = 0.98×0.98 mm, slice thickness = 1 mm, number of slices = 160), or a 3 T scanner (TR = 1900 ms, TE = 3.93 ms, flip angle = 15°, in-plane resolution = 1×1 mm, slice thickness = 1 mm, number of slices = 160).

2.3. Data processing & analysis

Measurements of whole brain volume (WBV), whole cerebellum volume (WCV), cerebellar GM volume, and cerebellar WM volume were collected by means of semiautomatic volumetric analysis. Analyze 10.0 © software [31] was used to obtain all volume measures, and all data were processed by a single trained observer (KKC). WBV was defined as the supratentorial cerebrum, infratentorial cerebellum and brainstem (midbrain, pons and medulla) not including the meninges (Fig. 1 A,B). A horizontal line was drawn across the plane of the foramen magnum to represent the inferior boundary of the whole brain volume. WCV was defined as the infratentorial cerebellum not including the brainstem (midbrain, pons and medulla), tentorium cerebelli or CSF; cerebellar GM volume was defined as the cortical tissue of the hemispheres and the vermis; cerebellar WM was defined as the arbor vitae and the superior, middle and inferior peduncles within the hemispheres (Fig. 1 C,D,E).

First, the semiautomatic analysis involves an automated seed-tool that segments regions of interest (ROIs) values based on intensity, and then a drawing tool is used to manually refine the borders of the ROIs originally defined by the automated tool. Anatomical features of the cerebellar peduncles were used as a guide to distinguish between cerebellum and brainstem. A consistent line was manually drawn on each of the images following the tissue boundary of 1) the midbrain to the superior cerebellar peduncle, 2) the pons to the middle cerebellar peduncle, and 3) the medulla to the inferior cerebellar peduncle; these boundaries were defined following Woolsey et al. [32]. Tentorium cerebelli and CSF were not included in any of the measures and were manually deleted from all ROIs.

In order to establish intra-rater reliability, three of the 40 images were randomly selected from the group of participants, blind to diagnosis. The cerebellum of each of the selected images was semi-automatically segmented three times as described above. Intra-rater reliability was assessed for each measure using a nested ANOVA in which the volume served as the dependent variable, subject and trial-within-subject were independent variables. No effect of trial was observed for any structure ($p = 0.21$ – 0.60). These values show that segmentation of the cerebellum was performed with a high degree of repeatability.

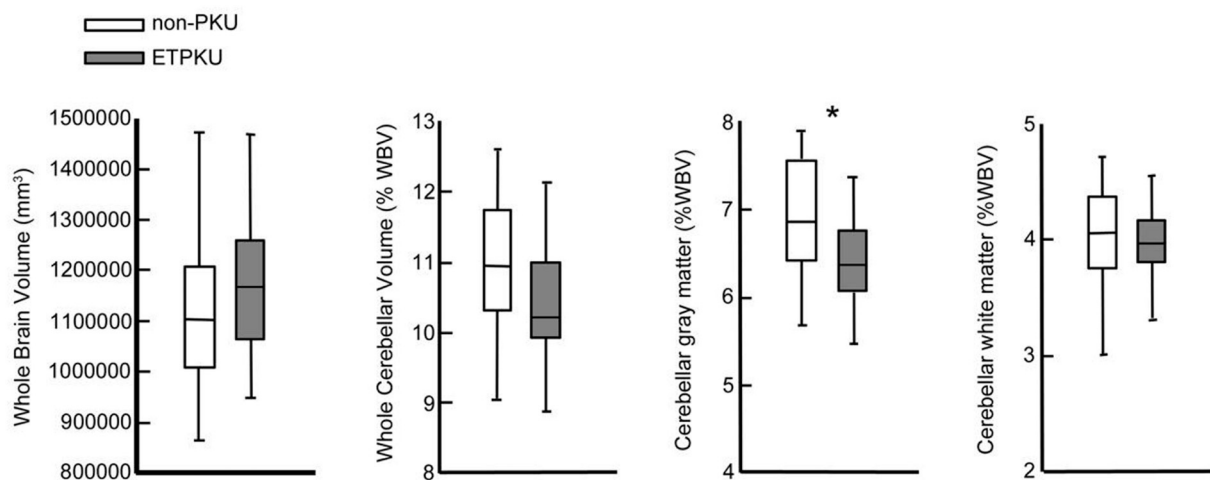


Fig. 2. Box and whisker plots of volume measures (mm³): WBV, WCV, cerebellar GM, and cerebellar WM in ETPKU and non-PKU groups. * Significant group difference ($p < 0.05$).

3. Results

Mean volumes for each structure are shown in Table 1 and Fig. 2.

3.1. Whole brain volume (WBV)

Hierarchical regression was employed to evaluate between-group differences in whole brain volume. This method allows evaluation of group-related differences in volume measures while accounting for the effects of individual differences in age. Whole brain volume served as the dependent variable. The first step of the analysis included age. The second step included group (ETPKU and non-PKU), with the age x group interaction included in the third (and final) step of the model. There were no significant effects of age or group (ETPKU and non-PKU) on whole brain volume (WBV) [$t(37) < 1.4$, $pr^2 < 0.05$, $p > 0.18$ in both instances]. The interaction between age and group was also not significant [$t(36) = 1.20$, $pr^2 = 0.04$, $p = 0.24$].

3.2. Cerebellar volumes

Hierarchical regression was employed to evaluate between-group differences in cerebellar volume measures, allowing evaluation of group-related differences in volume measures while controlling for the effects of individual differences in age and whole brain volume. In each hierarchical regression analysis conducted, the volume of the cerebellar structure (WCV, GM, WM) served as the dependent variable. In the first step of each analysis, age and WBV were included. The second step included group (ETPKU and non-PKU). The third step included all pairwise interactions (age x group, age x WBV, group x WBV), with the age x group x WBV interaction included in the final step of the model.

3.3. Whole cerebellar volume (WCV)

The effect of WBV on WCV was significant [$t(37) = 7.45$, $pr^2 = 0.60$, $p < 0.001$] while age was not [$t(37) = 1.58$, $pr^2 = 0.06$, $p = 0.12$]. After accounting for age and WBV, no effect of group was observed [$t(36) = 1.57$, $pr^2 = 0.06$, $p = 0.13$]. However, the age x group interaction approached significance [$t(33) = 1.78$, $pr^2 = 0.09$, $p = 0.085$] with WCV decreasing with age in the ETPKU group, while remaining stable in the non-PKU group. There were no significant effects of age x WBV [$t(33) < 1$, $pr^2 = 0.004$, $p = 0.71$], group x WBV [$t(33) = 1.63$, $pr^2 = 0.075$, $p = 0.11$], or age x group x WBV [$t(32) < 1$, $pr^2 = 0.002$, $p = 0.82$].

3.4. Cerebellar White matter (WM) volume

WBV contributed significantly to cerebellar WM volume [$t(37) = 5.73$, $pr^2 = 0.47$, $p < 0.001$], and the effect of age approached significance [$t(37) = 1.92$, $pr^2 = 0.09$, $p = 0.063$]. After accounting for variance related to WBV and age, there was no effect of group [$t(36) < 1$, $pr^2 < 0.001$, $p = 0.95$]. The effect of age x group interaction approached significance [$t(35) = 1.74$, $pr^2 = 0.08$, $p = 0.091$]. A significant decrease in WM volume was apparent when comparing younger (< 16 yrs) and older (> 16 yrs) participants with ETPKU ($M = 10.9\%$ and 10.0% of WBV, respectively) [$t(18) = 2.60$, $p = 0.018$]. In contrast, WM volume was comparable for younger and older participants in the non-PKU group ($M = 10.8\%$ and 11.2% of WBV, respectively) [$t(18) < 1$, $p = 0.38$]. There were no significant effects of age x WBV [$t(33) < 1$, $pr^2 < 0.001$, $p = 0.92$], group x WBV [$t(33) = 1.25$, $pr^2 = 0.05$, $p = 0.22$], or age x group x WBV [$t(32) < 1$, $pr^2 = 0.006$, $p = 0.65$].

3.5. Cerebellar Gray matter (GM) volume

The effect of WBV on cerebellar GM volume was significant [$t(37) = 6.19$, $pr^2 = 0.51$, $p < 0.001$], while age was not [$t(37) < 1$,

$pr^2 = 0.02$, $p = 0.36$]. After accounting for age and WBV, a significant effect of group was observed [$t(36) = 2.06$, $pr^2 = 0.11$, $p = 0.047$], with GM reduced in the ETPKU group ($M = 6.45\%$ of WBV) as compared to the non-PKU group ($M = 6.98\%$ of WBV). There were no significant effects of age x group [$t(33) = 1.18$, $pr^2 = 0.04$, $p = 0.25$], age x WBV [$t(33) < 1$, $pr^2 = 0.008$, $p = 0.60$], group x WBV [$t(33) = 1.29$, $pr^2 = 0.05$, $p = 0.21$], or age x group x WBV [$t(32) < 1$, $pr^2 = 0.009$, $p = 0.59$].

3.6. Relationship with blood Phe levels

To determine whether there was a relationship between mean blood Phe levels and brain volume measures in the ETPKU group, additional analyses were conducted. A series of analyses were performed examining mean lifetime blood Phe level, mean blood Phe level over the year prior to scanning, and mean blood Phe level over the previous three months prior to scanning. For analysis of cerebellar volumes, hierarchical regression analyses were employed, with age and WBV entered in the first step, the relevant blood Phe level (i.e., lifetime, past year, previous 3 months) in the second step, with interaction terms (age x WBV, age x relevant blood Phe level, WBV x relevant blood Phe level) in the third step.

3.6.1. Mean lifetime blood phe level

After accounting for age, mean lifetime blood Phe level contributed significantly to WBV [$t(13) = 2.61$, $pr^2 = 0.34$, $p = 0.02$]. Higher lifetime blood Phe level was associated with lower WBV. However, the interaction between lifetime Phe level and age did not have a significant effect on WBV [$t(12) < 1$, $pr^2 = 0.001$, $p = 0.93$].

After accounting for age [$t(13) = 2.42$, $pr^2 = 0.31$, $p = 0.03$] and WBV [$t(13) = 7.32$, $pr^2 = 0.81$, $p < 0.001$], there was no significant effect of lifetime Phe level [$t(12) = 1.05$, $pr^2 = 0.08$, $p = 0.31$] on WCV. Neither the interaction of lifetime Phe level with the age [$t(12) = 1.00$, $pr^2 = 0.10$, $p = 0.34$] or with WBV [$t(12) = 1.64$, $pr^2 = 0.23$, $p = 0.14$] reached significance. Analysis of cerebellar GM and WM volumes found no significant effects of lifetime Phe level [$t(12) < 1$, $pr^2 < 0.07$, $p > 0.40$ in both cases] or of any interaction effects [$t(11) < 1.5$, $pr^2 < 0.19$, $p > 0.18$ among the interactions].

3.6.2. Mean blood phe level over the previous year, and over the previous 3 months

The effects of mean blood Phe level over the last year and mean blood Phe level over the previous 3 months mirrored findings for mean lifetime blood Phe levels. Both measures of blood Phe levels contributed significantly to WBV after accounting for age [$t(16) > 2.2$, $pr^2 > 0.23$, $p < 0.05$ for both measures], with higher blood Phe levels associated with lower WBV. No other effects of blood Phe levels or interactions were significant [$p > 0.13$ in all cases].

4. Discussion

The present study extends past research on the neurological sequelae of ETPKU. Contrary to previous studies [29,33,34], WBV was not found to be significantly different in individuals with ETPKU as compared to non-PKU individuals. However, individuals with ETPKU had significantly reduced cerebellar GM volumes in comparison with non-ETPKU individuals. No significant group differences were observed in WCV or cerebellar WM volume.

The most consistent neuroanatomic finding in individuals with ETPKU is the presence of cerebral WM abnormalities characterized by hyperintensities on T2-weighted MR imaging and decreased mean diffusivity values using diffusion tensor imaging [35]. These abnormalities are observed primarily in the posterior periventricular area, extending anteriorly with increased severity. In terms of volume, Pérez-Dueñas and colleagues [33] found no differences in overall cerebral WM volume between individuals with and without ETPKU although, within

their ETPKU group, higher blood phe levels were associated with greater cerebral WM volume. Much less is known regarding the integrity of WM in the cerebellum, but previous studies suggest that WM abnormalities in the cerebellum may be less frequent [26–28]. This is generally in line with the present results showing no ETPKU-related differences in cerebellar WM volumes. However, we did find a non-significant trend ($p = 0.09$) towards an age-by-group interaction, with cerebellar WM volume decreasing with age in the ETPKU group, while remaining stable in the non-PKU group. A larger longitudinal study is needed to fully evaluate the extent to which this trend is trivial or not.

While the cortical WM abnormalities associated with ETPKU are quite pronounced, the present finding of decreased cerebellar GM volume in our ETPKU sample adds to a growing literature suggesting that cortical and subcortical GM structures may also be affected. For example, Bodner et al. [34] reported ETPKU-related differences in putamen volume. Christ et al. [36] documented decreased parietal and occipital GM volumes in a sample of individuals with ETPKU as compared to individuals without PKU. Most recently, Pilotto et al. [36] found that higher CSF phe levels were correlated with GM atrophy in right parietal and bilateral frontal regions in a small sample of individuals with ETPKU.

The current finding of ETPKU-related effects on cerebellar GM is also congruent with the findings from past research showing atypical cell counts and dendritic arborization of Purkinje cells of the cerebellum. Additionally, a number of studies of murine models of PKU have found decreased cerebellar weight, with the cerebellum actually more affected than the cortex [37,38]. Adelman et al. [39] reported cytological abnormalities in Purkinje and granular cells of the cerebellum. Additional studies have documented abnormalities in cell number, density, and/or dendritic arborization affecting multiple layers of the gray matter [38,40,41]. Across this work, the most consistent finding appears to be that of diminished dendritic arborization, particularly as it relates to reduced parallel fibers. It is unclear whether similar cellular-level disruptions in humans with ETPKU underlie the presently observed finding of reduced cerebellar GM volume. Future studies employing other approaches such as in vivo single-photon emission computerized tomography (SPECT) to examine receptor density and/or histological study of post-mortem brain tissue from individuals with ETPKU may provide insight in this question. [There are a handful of older histological studies of brain tissue in untreated PKU [23,24,42]; however, we unaware of any such studies of specimens from early-treated individuals.] Recent development of a large-animal model of PKU [43] also holds promise for bridging the gap between data from murine models and humans.

We found a significant association with WBV and dietary treatment adherence as reflected by blood Phe levels in the ETPKU group. However, we failed to find a significant association between any cerebellar volume measurement and dietary treatment adherence as reflected by blood Phe levels in the ETPKU group. No correlation was apparent with either lifetime or more recent (past year / past 3 months) Phe levels. It is possible that future studies employing a larger sample size (thus increased statistical power) and focusing on Phe levels during specific developmental epochs which are particularly relevant to cerebellum (e.g., birth to 2 years) may reveal relationships between cerebellar morphometry and Phe levels that were not evident due to the limitations of the current study. Additional longitudinal research is required to understand the complex relationship between Phe levels and neural development.

Despite the prevalence and severity of the cerebral WM abnormalities frequently observed in individuals with ETPKU, findings on the relationship between such abnormalities and clinical/functional outcome are equivocal. Whereas some studies have found a relationship between WM integrity and neuropsychological function [44–46], others have failed to do so [47,48]. Although speculative, it is possible that neurocognitive difficulties in ETPKU may be more closely tied to markers of GM disruption. Consistent with this, Bodner et al. [33]

reported a significant relationship between putamen volume and overall intellectual ability in a sample of individuals with ETPKU. Unfortunately, neuropsychological data was not available for the present sample of participants, and the extent to which the current finding of decreased cerebellar GM may relate to neurocognitive outcome remains unclear. Moving forward, it will be important to couple neuroimaging data collection with a comprehensive neuropsychological battery, including measures of motor learning and control (i.e., functions that rely heavily on cerebellum). Additional insight may also be gained by the application of functional MRI methods to evaluate the efficiency of neural processing in cerebellum and related brain regions.

4.1. Summary and conclusions

In summary, we found that individuals with ETPKU had reduced cerebellar GM volumes as compared to healthy non-PKU individuals. These findings expand on previous animal work suggesting that cerebellar GM is impacted by PKU. Additional studies are needed to fully elucidate the timing and extent of the impact of ETPKU on cerebellum and the associated neurocognitive consequences.

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References

- [1] J.V. Hughes, T.C. Johnson, Experimentally induced and natural recovery from the effects of phenylalanine on brain protein synthesis, *Biochim. Biophys. Acta* 517 (1978) 473–485.
- [2] G.S. Ribas, A. Sitta, M. Wajner, C.R. Vargas, Oxidative stress in phenylketonuria: what is the evidence? *Cell. Mol. Neurobiol.* 31 (2011) 653–662, <https://doi.org/10.1007/s10571-011-9693-2>.
- [3] F. Güttler, H. Lou, Dietary problems of phenylketonuria: effect on CNS transmitters and their possible role in behaviour and neuropsychological function, *J. Inher. Metab. Dis.* 9 (Suppl. 2) (1986) 169–177.
- [4] N.A. Holtzman, R.A. Kronmal, W. van Doorninck, C. Azen, R. Koch, Effect of age at loss of dietary control on intellectual performance and behavior of children with phenylketonuria, *N. Engl. J. Med.* 314 (1986) 593–598, <https://doi.org/10.1056/NEJM198603063141001>.
- [5] I. Smith, M.E. Lobascher, J.E. Stevenson, O.H. Wolff, H. Schmidt, S. Grubel-Kaiser, H. Bickel, Effect of stopping low-phenylalanine diet on intellectual progress of children with phenylketonuria, *Br. Med. J.* 2 (1978) 723–726.
- [6] R. Paine, The variability in manifestations of untreated patients with phenylketonuria (phenylpyruvic aciduria), *Pediatrics.* 20 (1957) 290–331.
- [7] D. Janzen, M. Nguyen, Beyond executive function: non-executive cognitive abilities in individuals with PKU, *Mol. Genet. Metab.* 99 (Suppl. 1) (2010) S47–S51, <https://doi.org/10.1016/j.ymgme.2009.10.009>.
- [8] S.E. Christ, S.C.J. Huijbregts, L.M.J. de Sonneville, D.A. White, Executive function in early-treated phenylketonuria: profile and underlying mechanisms, *Mol. Genet. Metab.* 99 (Suppl. 1) (2010) S22–S32, <https://doi.org/10.1016/j.ymgme.2009.10>.

- 007.
- [9] J. Weglage, M. Pietsch, B. Funders, H. Koch, K. Ullrich, Deficits in selective and sustained attention processes in early treated children with phenylketonuria - result of impaired frontal lobe functions? *Eur. J. Pediatr.* 155 (1996) 200–204.
- [10] R. Gassió, R. Artuch, M. Vilaseca, E. Fusté, C. Boix, A. Sans, J. Campistol, Cognitive functions in classic phenylketonuria and mild hyperphenylalaninaemia: experience in a paediatric population, *Dev. Med. Child Neurol.* 47 (2005) 443–448.
- [11] J. Pietz, R. Dunckelmann, A. Rupp, D. Rating, H.M. Meinck, H. Schmidt, H.J. Bremer, Neurological outcome in adult patients with early-treated phenylketonuria, *Eur. J. Pediatr.* 157 (1998) 824–830.
- [12] R. Gassió, E. Fusté, A. López-Sala, R. Artuch, M. Vilaseca, J. Campistol, School performance in early and continuously treated phenylketonuria, *Pediatr. Neurol.* 33 (2005) 267–271.
- [13] G.L. Arnold, B.M. Kramer, R.S. Kirby, P.B. Plumeau, E.M. Blakely, L.S. Sanger Cregan, P.W. Davidson, Factors affecting cognitive, motor, behavioral and executive functioning in children with phenylketonuria, *Acta Paediatr. Int. J. Paediatr.* 87 (1998) 565–570.
- [14] J. Weglage, M. Pietsch, B. Funders, H.G. Koch, K. Ullrich, Neurological findings in early treated phenylketonuria, *Acta Paediatr. Int. J. Paediatr.* 84 (1995) 411–415.
- [15] S.C.J. Huijbregts, F.J. Van Spronsen, R. Licht, L.M.J. De Sonneville, I.E. Berends, P.H. Verkerk, J.A. Sergeant, Motor function under lower and higher controlled processing demands in early and continuously treated phenylketonuria, *Neuropsychology.* 17 (2003) 369–379.
- [16] J.D. Schmahmann, J.C. Sherman, Cerebellar cognitive affective syndrome, *Int. Rev. Neurobiol.* 41 (1997) 433–440.
- [17] C.J. Stoodley, J.D. Schmahmann, Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing, *Cortex J. Devoted Stud. Nerv. Syst. Behav.* 46 (2010) 831–844, <https://doi.org/10.1016/j.cortex.2009.11.008>.
- [18] N. Ramnani, The primate cortico-cerebellar system: anatomy and function, *Nat. Rev. Neurosci.* 7 (2006) 511–522, <https://doi.org/10.1038/nrn1953>.
- [19] H. Baillieux, H.J. De Smet, P.F. Paquier, P.P. De Deyn, P. Mariën, Cerebellar neurocognition: insights into the bottom of the brain, *Clin. Neurol. Neurosurg.* 110 (2008) 763–773, <https://doi.org/10.1016/j.clineuro.2008.05.013>.
- [20] D.T. Stuss, *Principles of Frontal Lobe Function*, Oxford University Press, Oxford, UK, 2002.
- [21] S.E. Christ, A.J. Moffitt, D. Peck, Disruption of prefrontal function and connectivity in individuals with phenylketonuria, *Mol. Genet. Metab.* 99 (Suppl. 1) (2010) S33–S40, <https://doi.org/10.1016/j.ymgme.2009.09.014>.
- [22] S.E. Christ, A.J. Moffitt, D. Peck, D.A. White, The effects of tetrahydrobiopterin (BH4) treatment on brain function in individuals with phenylketonuria, *NeuroImage Clin.* 3 (2013) 539–547, <https://doi.org/10.1016/j.nicl.2013.08.012>.
- [23] E. Alvord, L. Stevenson, F. Vogel, R. Engle, Neuropathological findings in phenylpyruvic oligophrenia (phenylketonuria), *J. Neuropathol. Exp. Neurol.* 9 (1950) 298–310.
- [24] S. Kornguth, E. Gilbert-Barness, E. Langer, L. Hegstrand, Golgi-Kopsch silver study of the brain of a patient with untreated phenylketonuria, seizures, and cortical blindness, *Am. J. Med. Genet.* 44 (1992) 443–448, <https://doi.org/10.1002/ajmg.1320440412>.
- [25] C. Ficioglu, J.G. Dubroff, N. Thomas, P.R. Gallagher, J. Burfield, C. Husa, R. Randall, H. Zhuang, A pilot study of Fluorodeoxyglucose positron emission tomography findings in patients with phenylketonuria before and during Sapropterin supplementation, *J. Clin. Neurol. Seoul Korea.* 9 (2013) 151–156, <https://doi.org/10.3988/jcn.2013.9.3.151>.
- [26] M. Hájek, L. Hejzmanová, J. Prádný, Proton in vivo spectroscopy of patients with hyperphenylalaninaemia, *Neuropediatrics.* 24 (1993) 111–112.
- [27] J. Pietz, R. Kreis, H. Schmidt, U. Meyding-Lamade, A. Rupp, C. Boesch, Phenylketonuria: findings at MR imaging and localized in vivo H-1 MR spectroscopy of the brain in patients with early treatment, *Radiology.* 201 (1996) 413–420.
- [28] K.D. Pearsen, A.D. Gean-Marton, H.L. Levy, K.R. Davis, Phenylketonuria: MR imaging of the brain with clinical correlation, *Radiology.* 177 (1990) 437–440.
- [29] N.H. Pfandner, G. Reuner, J. Pietz, G. Jost, D. Rating, V.A. Magnotta, A. Mohr, B. Kress, K. Sartor, S. Hähnel, MR imaging-based volumetry in patients with early-treated phenylketonuria, *AJNR Am. J. Neuroradiol.* 26 (2005) 1681–1685.
- [30] D. Wechsler, Wechsler Abbreviated Scale of Intelligence, Psychological Corporation, San Antonio, TX, 1999.
- [31] R. Robb, D. Hanson, R. Karwoski, A. Larson, E. Workman, M. Stacy, Analyze: a comprehensive, operator-interactive software package for multidimensional medical image display and analysis, *Comput. Med. Imaging Graph.* 136 (1989) 433–454.
- [32] T.A. Woolsey, J. Hanaway, M.H. Gado, *The Brain Atlas: A Visual Guide to the Human Central Nervous System*, Third edition, John Wiley & Sons, Inc., Hoboken, NJ, 2008.
- [33] K.E. Bodner, K. Aldridge, A.J. Moffitt, D. Peck, D.A. White, S.E. Christ, A volumetric study of basal ganglia structures in individuals with early-treated phenylketonuria, *Mol. Genet. Metab.* 107 (2012) 302–307, <https://doi.org/10.1016/j.ymgme.2012.08.007>.
- [34] B. Pérez-Dueñas, J. Pujol, C. Soriano-Mas, H. Ortiz, R. Artuch, M.A. Vilaseca, J. Campistol, Global and regional volume changes in the brains of patients with phenylketonuria, *Neurology.* 66 (2006) 1074–1078, <https://doi.org/10.1212/01.wnl.0000204415.39853.4a>.
- [35] P.J. Anderson, V. Leuzzi, White matter pathology in phenylketonuria, *Mol. Genet. Metab.* 99 (Suppl. 1) (2010) S3–S9, <https://doi.org/10.1016/j.ymgme.2009.10.005>.
- [36] A. Pilotto, N. Blau, E. Leks, C. Schulte, C. Deuschl, C. Zipser, D. Piel, P. Freisinger, G. Gramer, S. Kölker, D. Haas, P. Burgard, P. Nawroth, H. Georg, K. Scheffler, D. Berg, F. Trefz, Cerebrospinal fluid biogenic amines depletion and brain atrophy in adult patients with phenylketonuria, *J. Inherit. Metab. Dis.* 42 (2019) 398–406, <https://doi.org/10.1002/jimd.12049>.
- [37] A.L. Prensky, M.A. Fishman, B. Daftari, Differential effects of hyperphenylalaninemia on the development of the brain in the rat, *Brain Res.* 33 (1971) 181–191.
- [38] O. Robain, H.M. Wisniewski, Y.H. Loo, G.Y. Wen, Experimental phenylketonuria: effect of phenylacetate intoxication on number of synapses in the cerebellar cortex of the rat, *Acta Neuropathol. (Berl.)* 61 (1983) 313–315.
- [39] L.S. Adelman, J.D. Mann, D.W. Caley, N.H. Bass, Neuronal lesions in the cerebellum following the administration of excess phenylalanine to neonatal rats, *J. Neuropathol. Exp. Neurol.* 32 (1973) 380–393.
- [40] O. Robain, G.Y. Wen, H.M. Wisniewski, J.W. Shek, Y.H. Loo, Purkinje cell dendritic development in experimental phenylketonuria. A quantitative analysis, *Acta Neuropathol.* 53 (1981) 107–112 (Berl.).
- [41] G. Huether, V. Neuhoff, R. Kaus, Brain development in experimental hyperphenylalaninaemia: disturbed proliferation and reduced cell numbers in the cerebellum, *Neuropediatrics.* 14 (1983) 12–19, <https://doi.org/10.1055/s-2008-1059547>.
- [42] N. Malamud, Neuropathology of phenylketonuria, *J. Neuropathol. Exp. Neurol.* 25 (1966) 254–268, <https://doi.org/10.1097/00005072-196604000-00006>.
- [43] E. Koppes, B. Redel, M. Johnson, K. Skvorak, L. Ghaloul-Gonzalez, M. Yates, D. Lewis, S. Gollin, Y. Wu, S.E. Christ, M. Yerle, A. Leshinski, L. Spate, J. Benne, S. Murphy, M. Samuel, E. Walters, S. Hansen, K. Wells, U. Lichter-Konecki, R. Wagner, J. Newsome, S.F. Dobrowolski, J. Vockley, R. Nicholls, A porcine model of phenylketonuria generated by CRISPR/Cas9 genome editing, *JCI Insight.* (in press).
- [44] P.J. Anderson, S. Wood, D. Francis, L. Coleman, V. Anderson, A. Boneh, Are neuropsychological impairments in children with early-treated phenylketonuria (PKU) related to white matter abnormalities or elevated phenylalanine levels? *Dev. Neuropsychol.* 32 (2007) 645–668.
- [45] H. Peng, D. Peck, D.A. White, S.E. Christ, Tract-based evaluation of white matter damage in individuals with early-treated phenylketonuria, *J. Inherit. Metab. Dis.* 37 (2014) 237–243, <https://doi.org/10.1007/s10545-013-9650-y>.
- [46] A. Hood, J. Rutlin, J.S. Shimony, D.K. Grange, D.A. White, Brain White matter integrity mediates the relationship between phenylalanine control and executive abilities in children with phenylketonuria, *JIMD Rep.* 33 (2017) 41–47, https://doi.org/10.1007/8904_2016_579.
- [47] M. Mastrangelo, F. Chiarotti, L. Berillo, C. Caputi, C. Carducci, C. Di Biasi, F. Manti, F. Nardecchia, V. Leuzzi, The outcome of white matter abnormalities in early treated phenylketonuric patients: a retrospective longitudinal long-term study, *Mol. Genet. Metab.* (2015), <https://doi.org/10.1016/j.ymgme.2015.08.005>.
- [48] D.A. White, J.A.V. Antenor-Dorsey, D.K. Grange, T. Hershey, J. Rutlin, J.S. Shimony, R.C. McKinstry, S.E. Christ, White matter integrity and executive abilities following treatment with tetrahydrobiopterin (BH4) in individuals with phenylketonuria, *Mol. Genet. Metab.* (2013), <https://doi.org/10.1016/j.ymgme.2013.07.010>.