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ORIGINAL ARTICLE

Decreased functional brain connectivity in individuals with early-treated phenylketonuria: evidence from resting state fMRI

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Abstract Previous histological and neuroimaging studies have documented structural abnormalities in the white matter of the brain in individuals with early-treated phenylketonuria (ETPKU). It remains unclear, however, the extent to which the function of the brain's interconnections are impacted by this condition. Presently, we utilized functional magnetic resonance imaging (fMRI) to evaluate the synchronization of neural signals (i.e., functional connectivity) among brain regions comprising the default mode network (DMN) in a sample of 11 individuals with ETPKU and 11 age- and gender-matched neurologically intact controls. The DMN is a group of interconnected brain regions that are known to be generally more active during rest than during task performance. Data analysis revealed decreased functional connectivity among DMN regions for the ETPKU group compared with the control group. Within the PKU group, we also found a significant relationship between blood phenylalanine (phe) levels and the functional connectivity between select regions of the DMN. In conjunction with findings from another recent fMRI study (Christ, Moffitt et al. 2010), the present results suggest that ETPKU-related deficiencies in functional connectivity are pervasive. The current findings also provide initial evidence that the extent of such impairment may be moderated in part by blood phe levels.

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Phenylketonuria (PKU) is an inborn error of metabolism characterized by a disruption in the metabolism of the amino acid phenylalanine (phe) into tyrosine. In individuals with PKU, the phenylalanine hydroxylase enzyme that is critical to this metabolic process is either absent or mutated, which results in decreased tyrosine production (Jervis 1953). In addition, excess phe competes with available tyrosine and other large neutral amino acids (e.g., tryptophan) to cross the blood-brain barrier. The accumulative consequences include decreased neurotransmitter production (e.g., dopamine, serotonin), oxidative stress, and disruption in protein synthesis (Güttler and Lou 1986; Hughes and Johnson 1978; Ribas et al. 2011). Individuals with PKU are identified at birth and immediately placed on a phe-restricted diet. Failure to impose and maintain dietary treatment results in severe injury to the developing nervous system, resulting in significant neurological and intellectual impairment (Paine 1957).

Although individuals with early-treated PKU (ETPKU) do not experience severe impairments, they do experience some neural and cognitive sequelae. ETPKU is associated with a slight decrease in overall intellectual abilities (see Brumm and Grant 2010 for review) coupled with circumscribed impairment in higher-order "executive" abilities (see Christ, Huijbregts et al. 2010 for review).

Neurologic findings in ETPKU

The most consistent neurologic finding in individuals with ETPKU is structural abnormalities in the white matter of the

brain (see Anderson and Leuzzi 2010 for review). The extent and severity of such abnormalities appear to be moderated by patient age and compliance with a phe-restricted diet (as reflected by blood phe levels), with older age and higher phe levels associated with increased white matter involvement (Anderson et al. 2004; Cleary et al. 1994).

Recent research in our laboratory (Christ, Moffitt et al. 2010) has begun to also evaluate the *functional* integrity of the brain and its neural connections in ETPKU. As a starting point, we utilized functional magnetic resonance imaging (fMRI) to study neural activation in a small sample of individuals with and without ETPKU while they performed an n-back working memory task. Analysis of the data revealed a number of brain regions, nearly half of which were located in the prefrontal cortex, that showed atypical neural activity in individuals with ETPKU compared with non-PKU individuals. In addition, we found ETPKU-related decreases in functional connectivity (i.e., the synchronization of neural activity) among a network of brain regions supporting working memory.

Of particular relevance to the present study, among those brain regions found to demonstrate atypical activation in individuals with ETPKU were several 'task negative' regions (i.e., regions known to typically show greater activation during baseline/rest than during task performance). These regions showed significantly less activation during the working memory task compared with the baseline task in the non-PKU group. In the ETPKU group, however, these regions showed little or no difference in activation between baseline and task conditions. These findings may point to an ETPKU-related disruption in the default mode network (DMN) of brain regions.

Default mode of brain function

The DMN consists of a group of interconnected brain regions that are generally more active when a person is at rest compared with when the person is performing a cognitive task (see van den Heuvel and Hulshoff Pol 2010 for review). The DMN includes regions in medial prefrontal cortex (MPFC), posterior cingulate cortex/precuneus cortex (PCC), and lateral parietal cortex (LPC; Fox et al. 2005). Converging evidence for the conceptualization of the DMN as a distinct brain network comes from fMRI studies of the spontaneous fluctuations in neural activity that are observed in the brain during periods of rest. Several such 'resting state' studies have documented a high degree of temporal correlation in spontaneous activity among the aforementioned DMN brain regions (e.g., Fox et al. 2005).

The precise function of the DMN is a topic of ongoing research; however, it has been hypothesized to play a role in (a) the representation and ongoing monitoring of one's external environment and internal (cognitive, emotional and physical) states (Gusnard and Raichle 2001; Shulman et al. 1997); (b) our experiences of unconstrained, stimulus independent thought (Binder et al. 1999; Shulman et al. 1997); (c) introspective mental activity including one's concept of "autobiographical" self and awareness of relation to the past, present, and future (Buckner and Carroll 2007); and/or (d) the shifting of the stream of consciousness from one's external reality to internally simulated experiences/scenarios (i.e., self-projection) to inform decision making and understanding of the mental states of others (i.e. theory of mind) (U. Frith and C. D. Frith 2003).

The present study

The goal of the present study was to evaluate the functional integrity of the DMN in individuals with ETPKU compared with healthy controls. We utilized fMRI to examine neural activity in the brain during rest (i.e., lying still with eyes open) and evaluated the degree of synchronization among these signals, with particular attention to known DMN regions. Based on prior reports of neurological abnormalities in individuals with ETPKU, we hypothesized that ETPKU would be associated with decreased functional connectivity among DMN regions.

Methods

Participants

Participants comprised a sample of 11 individuals (5 males, 6 females) with ETPKU ranging in age from 9 to 33 years (M=24.0, SD=8.6). For all individuals with ETPKU, diagnosis was made and phe-restricted dietary treatment was implemented shortly after birth, as indicated by medical records and/or patient report. Seven of the participants were classified as having classic PKU (phe levels >1200 μmol/L on normal diet) and the other four were classified as having moderate PKU (phe levels between 600-1200 μmol/L on normal diet). Regretfully, complete lifetime records of blood phe level could not be attained for several of the older participants in the sample. Recent blood phe levels, however, were available for all participants: The mean blood phe level over the year prior to testing was 697.1 μmol/L (SD= 425), and the mean level over the month prior to testing was 758.3 μmol/L (SD=441). None of the participants were being treated with sapropterin at the time of testing. Individuals with severe cognitive impairment or major medical disorders unrelated to PKU were excluded.

A sample of 11 neurologically uncompromised individuals (5 males, 6 females) ranging in age from 9 to 33 years

(M=23.0, SD=9.3) comprised an age- and gender-matched control group. Healthy non-PKU participants were recruited from the Columbia, Missouri, community.

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 115, with a mean of 99.6 (SD=11.5). For individuals in the control group, scores ranged from 102 to 120, with a mean of 112.1 (SD=6.7). The scores of the control group were significantly higher than those of the ETPKU group, $t(20)=3.11$, $p=.006$.

Neuroimaging data acquisition & analysis

The present study was approved by the University of Missouri-Columbia Internal Review Board. Scans were obtained on a 3 T Siemens Trio scanner with a standard 8-channel head coil. For alignment purposes, a set of structural images was collected first using a standard T1-weighted pulse sequence [MP-RAGE sequence: TR=2400 ms, TE=3.16 ms, flip angle=8°, in-plane resolution=1 x 1 mm, slice thickness=1 mm, number of slices=176]. For the resting state functional run, sets of 32 contiguous axial images (TR=2200 ms, TE=27, flip angle=90°, in-plane resolution=4.0 x 4.0 mm, slice thickness=4.0 mm) were acquired parallel to the anterior–posterior commissure plane; this procedure offered whole-brain coverage at a high signal-to-noise ratio. Each participant completed a 6-minute resting state run (164 acquisitions) during which time they were instructed to lie still with their eyes open.

Processing and analysis of resting state fMRI data

Functional imaging data were preprocessed and analyzed using BrainVoyager QX software (version 1.10; Brain Innovation, Maastricht, the Netherlands). Preprocessing steps included slice scan time correction, 3D motion correction, linear trend removal, transformation to standardized atlas space (Talairach and Tournoux 1988), and spatial smoothing (6 mm FWHM) to accommodate variations in activation loci across participants.

To evaluate functional connectivity among DMN brain regions, we conducted a seed-based correlation analysis (Fox et al. 2005). The fMRI time course data were extracted for seed regions centered in each of three major areas of the DMN: MPFC (-1, 47, -4), PCC (-5, -49, 40), and LPC (-45, -67, 36). The location and size (i.e., 12-mm-diameter spheres) of these seed regions were drawn from a previous meta-analytic study of functional connectivity and the DMN (Fox et al. 2005).

For each participant, a whole-brain correlation map was created by computing the correlation coefficient between a given seed region and the time course for all other brain voxels. The resulting set of correlations was then transformed using Fisher's r-to-z' transformation. [Fisher's transformation converts Pearson's r values to a normally distributed variable z' thus allowing the application of inferential statistical tests to the transformed values.] This approach was repeated separately for each seed region and each participant, in turn. Independent samples t tests were then used to compare functional connectivity (as reflected by the z' value) between the ETPKU and non-PKU groups for each seed region. For each of the resulting statistical maps, a corrected whole-brain p-value of 0.005 was achieved by employing a cluster size threshold based on Monte Carlo simulations (Forman et al. 1995; Goebel et al. 2006).

Results

Findings from the MPFC seed region analysis are shown in Fig. 1. As anticipated based on past research on the DMN (Fox et al. 2005), robust correlations were found between the fMRI signal fluctuations in the MPFC seed region and those in several DMN brain areas including PCC and LPC for the non-PKU group. A similar pattern of results, however, was not observed for the ETPKU group. In comparison with the non-PKU group, the PKU group demonstrated decreased functional connectivity between the MPFC seed region and several brain areas, most notably the PCC and LPC [$p<.05$ false discovery rate (FDR) corrected in all instances]. These findings are illustrated in the right panel of Fig. 1 and are described in Table 1.

Additional evidence for decreased functional connectivity in the PKU group comes from the PCC and LPC seed region analyses. ETPKU-related decreases in functional connectivity were observed between the PCC seed region and several brain areas including MPFC and LPC, and between the LPC seed region and several brain areas including MPFC and PCC ($p<.05$ FDR-corrected in all instances). Findings from these seed-based analyses are shown in Figs. 2 and 3, respectively, and described in Table 1. In addition, Fig. 4 shows results of a direct comparison of BOLD signal correlations among the three DMN seed regions (MPFC, PCC, and LPC).

Adherence to phe-restricted dietary treatment

Correlation analyses were conducted to evaluate the relationship between functional connectivity (as reflected by the z' value) and recent adherence to phe-restricted dietary treatment (as reflected by mean blood phe levels over the month prior to testing) within the ETPKU group. Findings are shown in Fig. 4. Mean phe levels negatively correlated with functional connectivity between the PCC and LPC seed

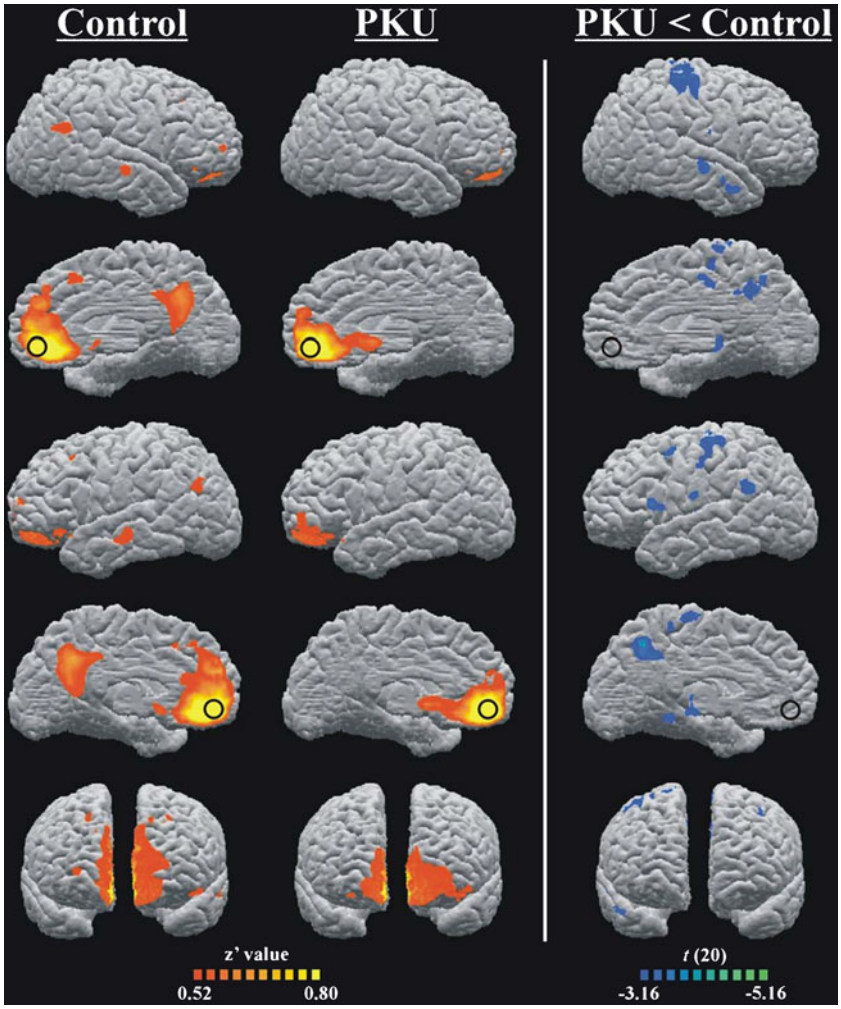

Fig. 1 Left and central panels: mean correlations between a seed region in MPFC (-1, 47, -4; marked by a black circle) and all other voxels in the brain shown separately for the PKU and non-PKU groups. Right panel: statistical map showing areas of significant functional connectivity differences between the two groups, $p<.005$ FDR-corrected

regions, $r=-.76$, p=.007. A similar (albeit slightly weaker) relationship was observed between phe levels and MFC-to-PCC connectivity, $r=-.58$, $p=.06$. Phe levels, however, were not correlated with MFC-to-LPC connectivity, $r=-.14$, $p=.68$.

Consistent with past studies of ETPKU and intellectual ability (Waisbren et al. 2007), higher phe levels were also associated with lower FSIQ scores, $r=-.72$, $p=.01$. No significant relationship was found between recent phe levels and age, $r=.52$, $p=.10$, or between FSIQ and age, $r=-.29$, $p=.39$, for the ETPKU group.

Age-related differences

Regression analyses were conducted to determine whether the observed PKU-related impairment in functional connectivity emerged, remained static, or resolved with increased age. Functional connectivity (as reflected by the z' value) between the MPFC and PCC seed regions served as the dependent variable. Age was included in the first step of the statistical model. Group was then entered into the second step of the model, followed by the interaction term (age x group) in the third step of the model. By utilizing this approach, we were able to evaluate whether the observed functional connectivity impairment varied as a function of age in individuals with ETPKU. The regression analysis was repeated with functional connectivity for each of the other region pairings (i.e., MPFC-to-LPC; PCC-to-LPC) serving as the dependent variable in turn.

Age did not explain a significant amount of variance in functional connectivity between MPFC and PCC, MPFC and LPC, or PCC and LPC, $\Delta R^2<.15$, $F(1, 20)<3.3$, $p>.08$ in all three instances. Furthermore, results indicated that, although functional connectivity between the MPFC and PCC regions was significantly decreased for the ETPKU group compared with the non-PKU group, $\Delta R^2=.40$, $F(1, 19)=13.6$, p=.002 (mean z' values of 0.22 and 0.62 for the ETPKU and non-PKU groups, respectively); there was no interaction between age and group, $\Delta R^2=.002$, $F(1, 18)<1$, p=.82. Similarly, a significant main effect of group for functional connectivity between the PCC and LPC regions was apparent, $\Delta R^2=.34$, $F(1, 19)=11.1$, p=.004 (mean z' values of 0.21 and 0.59 for the ETPKU and non-PKU groups, respectively), but an interaction

Table 1 Regions exhibiting group-related differences (PKU<Control) in functional connectivity with DMN seed regions*

Region	Location	BA	Peak activation			Volume	
			x	y	z	(mm^3)	*t* value†
Medial prefrontal cortex seed region (-1, 47, 4)							
1	Right middle temporal gyrus	22	53	-11	-6	578	3.46
2	Right anterior superior temporal gyrus	38	44	10	-21	429	3.41
3	Right insula	13	35	-8	18	339	3.28
4	Right precentral gyrus / postcentral gyrus	3/4	35	-32	57	4946	3.56
5	Right thalamus / putamen	NA	26	-26	0	1222	3.59
6	Right posterior cingulate cortex	24	11	-20	39	587	3.34
7	Left precuneus	7	-4	-56	42	3825	3.64
8	Left medial frontal gyrus	6	-7	-23	63	674	3.43
9	Left thalamus / brainstem	NA	-4	-29	-12	2639	3.40
10	Left parahippocampal gyrus / fusiform gyrus	36/37	-25	-41	-9	395	3.37
11	Left insula	13	-34	1	21	690	3.54
12	Left precentral gyrus / postcentral gyrus	3/4	-37	-23	54	1706	3.49
13	Left inferior frontal gyrus	44	-49	16	12	318	3.45
14	Left inferior parietal lobule	40	-55	-47	24	715	3.45
Posterior cingulate/precuneus seed region (-5, -49, 40)							
1	Right angular gyrus	39	50	-62	30	1003	3.72
2	Bilateral medial frontal gyrus	9	-4	43	15	1594	3.36
3	Left ventral medial frontal gyrus	9/10	-4	49	0	1133	3.60
4	Left dorsal medial frontal gyrus	8	-1	34	39	458	3.45
5	Left angular gyrus	39	-49	-68	21	2559	3.78
Lateral parietal cortex Seed region (-45, -67, 36)							
1	Right middle temporal gyrus	22	56	-38	3	420	3.41
2	Right middle temporal gyrus / inferior temporal gyrus	20/21	56	-23	-12	1005	3.46
3	Right middle temporal gyrus	22	53	-11	-6	315	3.32
4	Right anterior prefrontal white matter	NA	26	34	0	422	3.53
5	Right middle frontal gyrus	6	23	4	39	355	3.41
6	Bilateral anterior cingulate cortex	24	11	28	6	2855	3.62
7	Right medial frontal gyrus / anterior cingulate	9/32	8	31	33	1273	3.36
8	Bilateral precuneus / posterior cingulate	7/31	-10	-53	42	3639	3.54
9	Right medial frontal gyrus / anterior cingulate	9/32	5	37	24	373	3.26
10	Right cerebellum	NA	8	-86	-18	722	3.46
11	Left anterior cingulate cortex	32	-16	25	33	522	3.29
12	Left anterior prefrontal white matter	NA	-25	28	15	325	3.40
13	Left inferior frontal gyrus / anterior insula	9/13	-37	7	18	834	3.46

Notes. NA: not applicable. BA: approximate Brodmann's Area

*No regions exhibited PKU-related increases (PKU>control) in functional connectivity with the seed regions. In addition, only regions in excess of 300 mm^3 in size are listed

† Degrees of freedom=20; $p<.005$ false discovery rate-corrected in all instances

between age and group was not, $\Delta R^2=.02$, $F(1, 18)<1$, $p=.41$. Finally, the main effect of group for MPFC-to-LPC connectivity approached significance, $\Delta R^2=.13$, $F(1, 19)=3.5$, $p=.07$ (mean z' values of 0.18 and 0.43 for the ETPKU and non-PKU groups, respectively); but the interaction did not, $\Delta R^2=.005$, $F(1, 18)<1$, $p=.72$. These results suggest that ETPKU-related deficiencies in functional connectivity are static across the age range of the present sample (i.e., 9 to 33 years of age).

Relationship to full scale IQ

Additional regression analyses were conducted to determine the extent to which functional connectivity measures

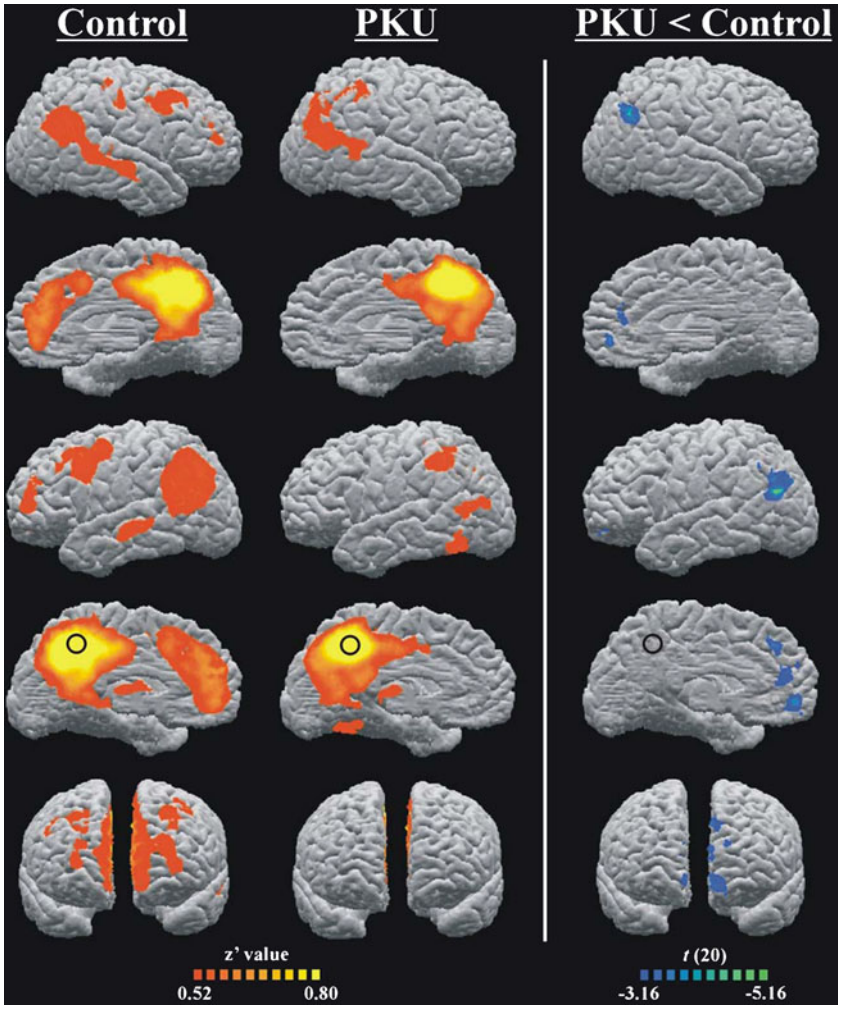

Fig. 2 Left and central panels: mean correlations between a seed region in PCC (-5, -49, 40; marked by a black circle) and all other voxels in the brain shown separately for the PKU and non-PKU groups. Right panel: statistical map showing areas of significant functional connectivity differences between the two groups, $p<.005$ FDR-corrected

explain variance in overall intellectual ability. FSIQ served as the dependent variable. Group was entered in the first step of the statistical model, followed by functional connectivity (as reflected by the z' value) between the MPFC and PCC seed regions in the second step of the model. The interaction term (group x functional connectivity) was entered in the third step of the model. The regression analysis was repeated with functional connectivity for each of the other region pairings (i.e., MPFC-to-LPC; PCC-to-LPC).

Results revealed no significant relationship between FSIQ and functional connectivity among any of the three region pairings, $\Delta R^2<.07$, $F(1, 19)<2.2$, $p>.15$ in all instances. The interactions (group x functional connectivity) also failed to approach significance, $\Delta R^2<.05$, $F(1, 18)<1.4$, $p>.26$ in all instances.

Relationships among functional connectivity, recent Phe levels, and FSIQ

As noted earlier, recent blood phe levels were negatively correlated with both FSIQ ($r=-.72$, $p=.01$) and PCC-to-LPC functional connectivity ($r=-.76$, $p=.007$). However, FSIQ and PCC-to-LPC functional connectivity were not related, $\Delta R^2=.07$, $F(1, 19)=2.2$, $p=.16$. Taken together, these results suggest that the relationship between blood phe levels and FSIQ may be unique from the relationship between blood phe levels and PCC-to-LPC functional connectivity. This finding was further confirmed via a regression analysis in which both FSIQ and PCC-to-LPC connectivity were simultaneously regressed on blood phe level ($sr^2=0.17$ and 0.23, respectively), $t(8)>2.3$, $p<.05$ in both instances.

Discussion

A recent functional neuroimaging study (Christ, Moffitt et al. 2010) documented decreased functional connectivity among brain regions in individuals with ETPKU during performance of a working memory task. Building upon this research, the current study was designed to further evaluate functional connectivity in ETPKU, specifically as it relates to the DMN, a network of brain regions that are more active during periods of rest compared with periods of task performance. Results of the present study are consistent with the

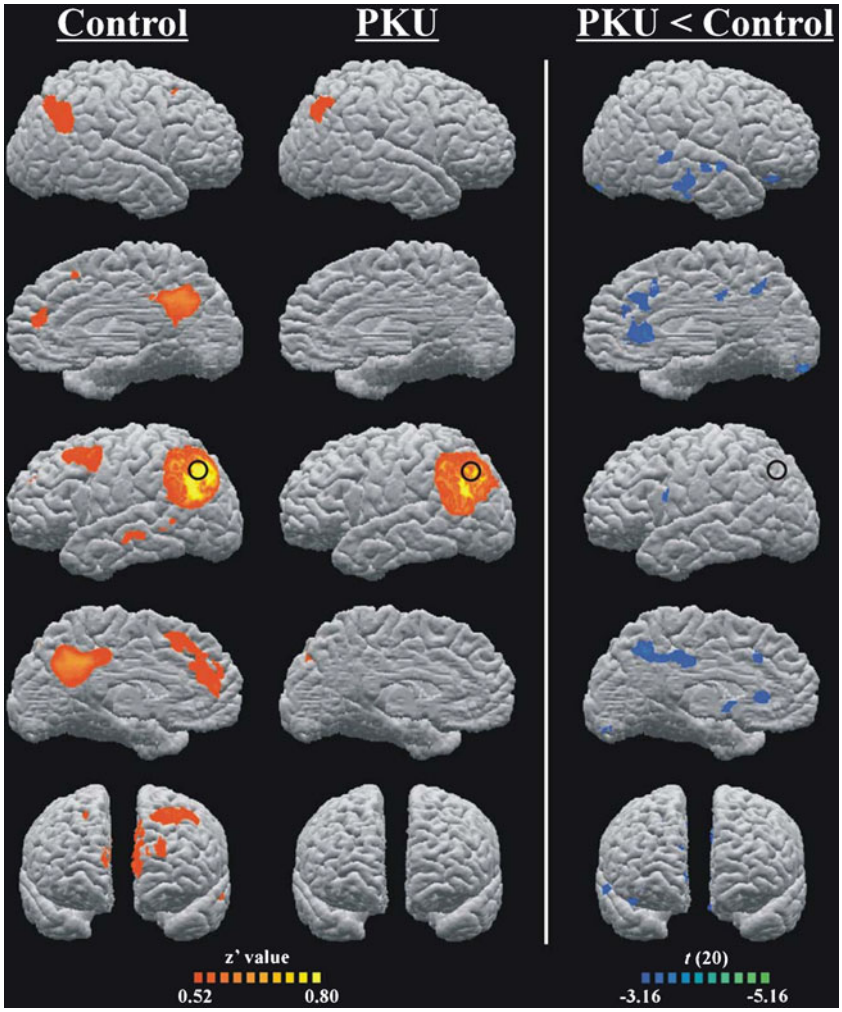

Fig. 3 Left and central panels: mean correlations between a seed region in LPC (-45, -67, 36; marked by a black circle) and all other voxels in the brain shown separately for the PKU and non-PKU groups. Right panel: statistical map showing areas of significant functional connectivity differences between the two groups, $p<.005$ FDR-corrected

hypothesis that ETPKU is associated with impairments in functional connectivity. Further, these results suggest that the degree of impairment is moderated by adherence to phe-restricted dietary treatment (as reflected by blood phe levels).

As described previously, the present study utilized a seed-based approach wherein we systematically evaluated synchronization of neural activity (i.e., functional connectivity) among three 'core' DMN brain regions (i.e., MPFC, PCC, & LPC) as well as between each of these three regions and the remainder of the brain. Consistent with previous research involving neurologically uncompromised individuals (e.g., Fox et al. 2005), analysis of data for our non-PKU group revealed a robust correlation in neural activity among these three regions and other known DMN brain regions (e.g., inferior temporal gyrus; parahippocampal gyrus) during resting state. A similar pattern, however, was not observed for the ETPKU group. Compared with the non-PKU group, the ETPKU group demonstrated decreased functional connectivity between DMN brain regions. This finding replicates our previous report of an ETPKU-related decrease in functional connectivity (Christ, Moffitt et al. 2010) and suggests that both 'task positive' and 'task negative' brain networks are affected.

Findings from many of the early post-mortem and neuroimaging studies on ETPKU pointed to posterior aspects of the cortex as the primary locus of injury in individuals with ETPKU (e.g., Thompson et al. 1990). This has long stood in apparent contradiction, however, to findings from behavioral studies suggesting that abilities subserved by the PFC (e.g., working memory and inhibition) are most affected by ETPKU (see Christ, Huijbregts et al. 2010 for review). Recently, by using more sophisticated data collection and analysis methods, neuroimaging studies have provided increasing evidence supporting the presence of prefrontal involvement in ETPKU. For example, a diffusion tensor imaging (DTI) study by White et al. (2010) found microstructural abnormalities in anterior, but not posterior, corpus callosum. Additional support comes from our earlier fMRI study (Christ, Moffitt et al. 2010), which documented atypical brain activation and/or decreased functional connectivity in several regions of PFC. The present finding of

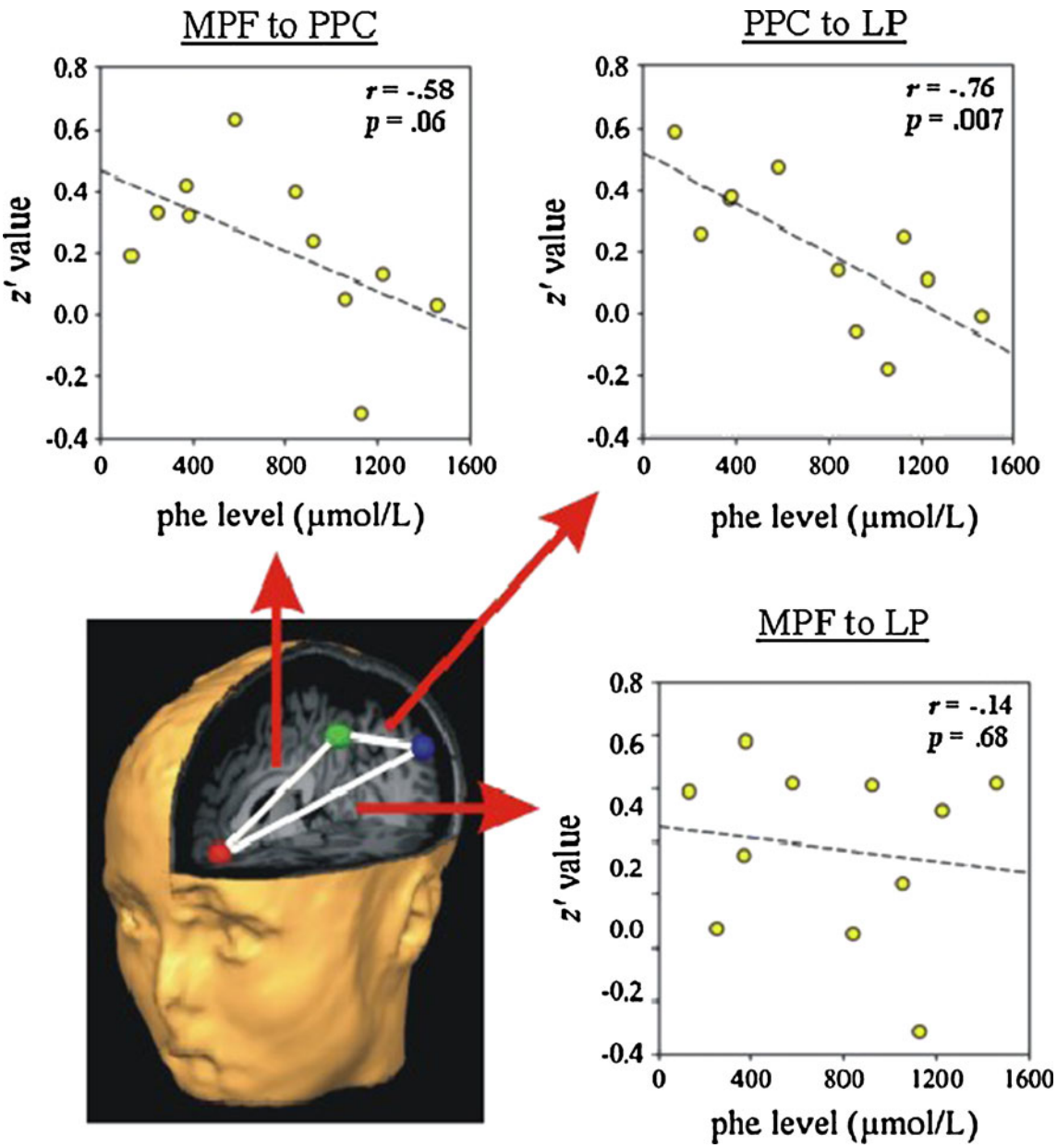

Fig. 4 Results of correlation analysis between recent dietary control (as reflected by mean blood phe levels over the month prior to testing) and functional connectivity (as reflected by the z' value) among seed regions in MPFC (-1, 47, -4), PCC (-5, -49, 40), and LPC (-45, -67, 36). [Note that, like the Pearson's *r* statistic, the z' statistic represents that strength of the linear relationship between two variables (e.g., neural activity in one region relative to another), with higher absolute values representing a stronger relationship. Also similarly, the sign (+/-) of the z' statistic reflects whether the two variables are positively or negatively related]

decreased functional connectivity between MPFC and more posterior DMN regions is also consistent with the hypothesis that prefrontal regions are affected in ETPKU.

In the current study, we also observed a significant relationship between compliance with phe-restricted dietary treatment and the extent of disruption in functional connectivity. ETPKU participants with higher blood phe levels (reflecting poorer dietary compliance) showed decreased neural synchronization within the DMN compared with ETPKU participants with lower phe levels. In fact, as can be seen in Fig. 4, a few participants with higher phe levels actually showed a slight *negative* correlation (reflected by a negative z' value) between DMN regions that one would otherwise expect to be strongly positively correlated. This finding introduces the possibility that very high phe levels may be associated with qualitative shift in the topological organization of brain networks such as the DMN. Moving forward, the collection of larger participant samples may

allow for the application of graph theory (Wang et al. 2010) and other sophisticated analysis techniques to directly examine this possibility and others.

The present finding of a relationship between phe levels and functional connectivity is consistent with a past structural MRI study by Cleary et al. (1994) that documented an association between concurrent phe levels and the severity of white matter injury in individuals with ETPKU. Importantly, in a follow-up study, Clearly et al. (1995) found reduced white matter abnormalities in a subset of patients following return to strict dietary control. Additional research is necessary to evaluate whether the presently observed impairments in functional connectivity are static or resolve with improved dietary compliance.

Additional limitations

The present sample size was comparable to those used in past ETPKU neuroimaging studies and provided sufficient statistical power to detect group-related differences in functional connectivity as well as a relationship between phe levels and connectivity. Another concern with smaller sample sizes, however, is the ability to generalize findings to the broader population. Within this context, it is worthy to note that the participant sample, methodology, and scanner site utilized in the present study were distinct from those employed in our previous fMRI study (Christ, Moffitt et al. 2010: 1.5 T MRI scanner; present study: 3 T MRI scanner). Despite these differences, both studies found ETPKU-related impairments in functional brain connectivity, supporting the generalizability of this finding. Regardless, additional research employing a larger sample size will be critical to further document the pervasiveness of the observed impairment and to delineate the extent to which such impairments are moderated by factors such as age. Future, comprehensive studies will also be important to understand the relationship between functional connectivity deficiencies and previous reports of structural white matter abnormalities and cognitive behavioral impairments in individuals with ETPKU.

Additional research is also needed to better understand the inter-relationship among blood phe levels, functional brain connectivity, and the cognitive and behavioral difficulties associated with ETPKU. The present study found that whereas recent blood phe levels were correlated with both FSIQ and functional brain connectivity, FSIQ and functional connectivity were not correlated with each other. One possible explanation is that the current IQ test (i.e., Wechsler Abbreviated Scale of Intelligence) may not have fully captured those aspects of cognitive functioning (e.g., executive abilities, episodic memory) that are most sensitive to individual differences in functional connectivity within the DMN (Hampson et al. 2006). Inclusion of a more comprehensive neuropsychological assessment battery in future studies of ETPKU and functional brain connectivity would be helpful.

As noted earlier, unfortunately, complete lifetime records of blood phe levels were not available for several of the older participants with ETPKU in the current study. As a consequence, the extent to which phe levels during other epochs of development (e.g., early childhood) contributed to the observed impairment remains unclear. Similarly, insufficient treatment data prevented us from evaluating the sensitivity of functional connectivity to individual differences in the ratio of blood phe to tyrosine (phe:tyr), which has been proposed as a superior alternate to blood phe levels as a biological marker of PKU (e.g., Sharman et al. 2010). In the future, we hope to address these issues and others by extending our program of research to a larger participant sample that is better characterized.

Lastly, despite its wide establishment within the neuroscience field as a marker of neural connectivity (e.g., Greicius et al. 2009; Mazoyer et al. 2001; Shulman et al. 1997), fMRI connectivity analysis (like any other approach) has potential limitations. For example, fMRI methodology does not directly measure neural activity, rather it measures a *correlate* of neural activity, namely the local changes in blood flow and the ratio of oxygenated to deoxygenated blood (termed the blood-oxygen level dependent or BOLD response) that accompany changes in neural activity. Within this context, it remains unknown to what extent the presence of vascular abnormalities (related to a given disease state) might impact our ability to assess neural activity and connectivity based on observed changes in BOLD response (e.g., Holodny et al. 1999). Moving forward, applications of other methodologies (e.g., electroencephalography, DTI) in conjunction with fMRI will be important in validating the present findings of disrupted functional connectivity in ETPKU and elucidating how such functional disruptions are related to the structural white matter abnormalities often also experienced by individuals with ETPKU.

Summary & conclusions

Taken with findings from another recent fMRI study (Christ, Moffitt et al. 2010), the present results suggest that ETPKU is associated with deficiencies in functional connectivity affecting both task-positive and task-negative brain networks. The current findings also provide initial evidence that the extent of such impairment may be moderated by compliance with phe-restricted dietary treatment. Additional research is needed, however, to better understand the link between the observed decrease in functional connectivity and the cognitive and behavioral difficulties associated with ETPKU. Finally, future inclusion of a longitudinal component will allow for study of when and how impairments in

functional connectivity change as a function of age and/or treatment intervention.

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