Brain Structural Alterations in Obese Children With and Without Prader-Willi Syndrome

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Abstract: Prader-Willi syndrome (PWS) is a genetic imprinting disorder that is mainly characterized by hyperphagia and childhood obesity. Previous neuroimaging studies revealed that there is a significant difference in brain activation patterns between obese children with and without PWS. However, whether there are differences in the brain structure of obese children with and without PWS remains elusive. In the current study, we used T1-weighted and diffusion tensor magnetic resonance imaging to investigate alterations in the brain structure, such as the cortical volume and white matter integrity, in relation to this eating disorder in 12 children with PWS, 18 obese children without PWS (OB) and 18 healthy controls. Compared with the controls, both the PWS and OB groups exhibited alterations in cortical volume, with similar deficit patterns in 10 co-varying brain regions in the bilateral dorsolateral and medial prefrontal cortices, right anterior cingulate cortex, and bilateral temporal lobe. The white matter integrities of the above regions were then examined with an analysis method based on probabilistic tractography. The PWS group exhibited distinct changes in the reduced fractional anisotropy of white matter fibers connected to the co-varying regions, whereas the OB group did not. Our findings indicated that PWS and OB share similar gray matter alterations that are responsible for the development of eating disorders. Additionally, the distinct white matter alterations might explain

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Key words: childhood obesity; Prader-Willi syndrome; eating disorder; cortical volume; tractography

INTRODUCTION

The rate of childhood obesity is an increasing worldwide concern because more than one-third of children and adolescents are overweight or obese [Ogden et al., 2014]. Childhood obesity can lead to serious health problems in terms of both immediate and long-term effects; 70% of obese youth exhibit at least one risk factor for cardiovascular disease [Freedman et al., 2007], and obese children are also likely to remain obese when they reach adulthood [Guo and Chumlea, 1999]. In response to rising childhood obesity issues, recent research has identified the brain circuitry involved in motivation, reward, and cognitive control in obese children [Bruce et al., 2010].

Prader-Willi syndrome (PWS), which is one of a very few human genetic models for studying obesity [Holland et al., 2003], results in extreme hyperphagia and early childhood onset obesity caused by excessive and pathological overeating and food reinforcement [Butler, 1990]. Early studies compared PWS and obese children (OB) in terms of body composition, obesity-related variables [Kennedy et al., 2006; Theodoro et al., 2006], energy consumption [Butler et al., 2007], and biochemical measurements [Haqq et al., 2003; Proto et al., 2007]. These findings suggested that PWS and OB share similar symptoms, but PWS can be more serious in terms of the disruption of satiety mechanisms.

Moreover, emerging functional neuroimaging evidence indicates the presence of hyper-responsive neural networks that involve brain regions, such as the hypothalamus, ventromedial prefrontal cortex, and insula, as well as heightened reward circuitry activation in PWS in response to food stimulation [Dimitropoulos et al., 2006; Holsen et al., 2006; Mantoulan et al., 2011; Miller et al., 2007; Moran and Westerterp-Plantenga, 2012; Ogura et al., 2013; Shapira et al., 2005]. Compared with body mass index (BMI)-matched non-PWS obese subjects, PWS subjects exhibited hyper-activations in the subcortical reward circuitry and hypo-activation in cortical inhibitory regions [Holsen et al., 2012]. Non-food-stimulated resting-state studies have also implicated a deregulation of energy intake and impairment in inhibitory control in PWS [Zhang et al., 2013, 2015]. Only a few studies have focused on anatomical alterations in PWS [Lukoshe et al., 2014; Ogura et al., 2011; Yamada et al., 2006]. Compared with controls, PWS subjects exhibited smaller gray matter volumes [Ogura et al., 2011], reduced cortical complexity [Lukoshe et al., 2014], and decreased white matter integrity [Yamada et al., 2006].

Despite this recent progress in functional and structural neuroimaging studies of PWS and OB, no three-group

(PWS, OB, and control) experiment has been designed to explore brain structural changes in PWS compared with OB subjects. The current study aims to identify the neural mechanisms associated with childhood obesity that are involved in dietary restraint and extreme hyperphagia observed in PWS via comparisons between PWS, OB, and control subjects. We hypothesized that the PWS and OB groups would exhibit similar alterations in brain structures that are associated with cognitive-emotional regulation and food-intake control compared with controls. We further hypothesized that the changes would be more prominent in PWS subjects than OB subjects, which would explain the symptoms associated with food intake in PWS, including excessive hyperphagia and constant hunger. The cortical volume and white matter integrity (measured by fractional anisotropy [FA]) are among the most important quantifications for exploring brain structural characteristics. In addition, because the hypothalamus is crucial for hunger, we assessed the gray matter volume (GMV) among the three groups via ROI analysis. To test our hypotheses, we performed T1-weighted magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) in a three-group study that included PWS, OB, and control subjects.

METHODS

Subjects

Twelve children with PWS (8 girls and 4 boys; mean age = 7.2 years), 18 obese children (12 girls and 6 boys; mean age = 9.0 years), and 18 healthy, normal-weight children (12 girls and 6 boys; mean age = 8.3 years) participated in this study. The three groups were age- and gender-matched ($P > 0.05$), as illustrated in Table I. BMI-z were used to evaluate the subjects' weight level. The OB subjects were recruited based on a BMI percentile $> 95\%$. All PWS subjects were first characterized by DNA methylation analysis at the 5' *SNURF-SNRPN* locus and by fluorescence in situ hybridization (FISH) using the *SNURF-SNRPN* probe and a distal chromosome 15 control probe. If a subject was positive for PWS according to DNA methylation analysis and had an intact 15q11-q13 region according to FISH, a DNA polymorphism analysis was used to distinguish maternal UPD 15 from an imprinting defect. None of the patients with PWS were currently at the stage of receiving growth hormone therapy or estrogen/androgen replacement therapy when recruited for the MRI scans. The control group consisted of 18 healthy and normal weight subjects, including siblings of the PWS children. The overall research protocol was

TABLE I. Demographic information for the PWS, OB, and control groups

Variable	Group			T value			P value		
	Control	PWS	OB	A	B	C	A	B	C
Gender (Boys/Girls)	6/12	4/8	6/12	0.00	0.00	0.00	1.000	1.000	1.000
Age (years)	8.3 ± 0.9	7.2 ± 1.2	9.0 ± 0.9	0.74	−0.54	−1.18	0.466	0.594	0.252
BMI-z	0.79 ± 0.21	2.16 ± 0.25	3.06 ± 0.19	−6.16	−8.22	−2.65	0.000	0.000	0.014

Values are given as the means ± standard error.

A: Comparison between the PWS and control groups (control - PWS).

B: Comparison between the OB and control groups (control - OB).

C: Comparison between the PWS and OB groups (PWS - OB).

approved by the Institutional Review Board at the University of Florida, and informed consent was obtained from each participant and/or from his/her legal guardian.

Image Acquisition

T1-weighted MRI

The experiments were performed on a 3.0 T head-dedicated Siemens Allegra MRI scanner (Siemens, Munich, Germany). A set of T1-weighted, high-resolution structural images was acquired using a magnetization prepared rapid acquisition gradient echo sequence with the following imaging parameters: matrix size = 512×512 , field of view = 240×240 mm², slice thickness = 1.1–1.4 mm, TR = 1,500 ms, TE = 4.38 ms, and flip angle = 8°.

Diffusion tensor imaging

DTI images were acquired with the same MRI instrument and after the T1-weighted MRI scans. The sequence parameters were as follows: matrix size = 128×128 , field of view = 220.16×220.16 mm², slice thickness = 4 mm, TR = 4,400 ms, and TE = 94 ms. The diffusion sensitizing gradients were applied along 24 non-collinear directions (12 directions with $b = 750$ s/mm² and 12 directions with $b = 1,500$ s/mm²), with one acquisition without diffusion weighting ($b = 0$ s/mm²).

Image Processing and Measurement

Gray matter analysis

The cortical volume was analyzed using the Freesurfer package [Fischl and Dale, 2000], which is well documented at <http://surfer.nmr.mgh.harvard.edu>. First, the T1-weighted images were automatically processed via the following steps: motion correction, removal of non-brain tissue, transformation to Talairach-like space, segmentation of gray and white matter, intensity normalization, tessellation of the gray and white matter boundaries, automated topology correction, and surface deformation following intensity gradients to optimally place the gray/white and gray/CSF borders that most

accurately defined the transitions between tissues. Second, each cortical measurement of each vertex of the subjects' surfaces was mapped on a common spherical coordinate system using a spherical transformation. Next, the results were smoothed using a Gaussian kernel of 10 mm.

To explore the anatomical alterations in PWS and OB, the cortical volume differences were estimated using the general linear model with contrasts for PWS versus control, OB versus Control, and PWS versus OB in a vertex-wise manner while controlling for age and gender. Statistical significance was defined in two ways. For differences between PWS and OB (results in PWS vs. OB), the significance in PWS versus OB was evaluated using a cluster-wise P (CWP) value correction procedure for multiple comparisons with cluster-based Monte Carlo simulation using 5,000 permutations. Clusters with CWP value < 0.05 were considered statistically significant [Kamagata et al., 2017]. For similarities between PWS and OB (overlapped results in PWS vs. control and OB vs. control), we first acquired the exploratory results ($P < 0.05$ at the vertex level, uncorrected) [Nenadic et al., 2017; Reijmer et al., 2017] in PWS versus control and OB versus control respectively, and then defined the clusters in these overlapped results with size > 200 mm³ as significant results. This was because the clusters in these overlapped results met two criteria ($P < 0.05$ in PWS vs. control and $P < 0.05$ in OB vs. control), and their joint P value could be considered much smaller than 0.05 [Chang et al., 2017]. Moreover, we ruled out regions under 200 mm³ for further correction. Finally, the clusters with significance that co-varied in PWS and OB subjects were defined as ROIs, while the other regions in the results of PWS versus control and OB versus control were not investigated in this study. ROIs were then used as seeds for further tractography analysis. In addition, these ROIs were also verified with the CWP correction which was the same as the procedure in PWS versus OB.

Tractography analysis

To further investigate the white matter fibers that were connected to the ROIs and then compare the FA of the fibers between the PWS, OB, and control groups, an analysis method was applied based on probabilistic tractography. The

Figure 1.

The flow chart of the white matter analysis for a sample ROI. The analysis method based on tractography illustrates how white matter fiber seedings from a sample ROI that were significant across subjects were identified. [Color figure can be viewed at wileyonlinelibrary.com]

procedure was performed with FMRIB's diffusion toolbox [Behrens et al., 2007] of FSL [Smith et al., 2004], which is a library of image analysis and statistical tools for fMRI, MRI, and DTI brain imaging data (<http://www.fmrib.ox.ac.uk/fsl/>). The flow chart of this process for a sample ROI is presented in Figure 1.

During preprocessing, we used affine and nonlinear transforms to convert the ROIs into individual diffusion spaces that were set as seeds. Next, 5,000 streamlines were drawn from the connectivity distributions of each seed voxel, which yielded a connectivity map that recorded the locations of the voxels and numbers of streamline crossings. Ultimately, we thresholded these voxels with a probability $> 1\%$ to obtain the tracts that were connected to the ROIs.

Notably, preprocessing was performed in individual diffusion spaces, and the results from the same ROIs were different across subjects (an example of the fibers of subjects 1 to 3 is shown in Fig. 1), which is natural and essential for the uniqueness of human connectomes [Sporns et al., 2005] and variations in human cognition [Boorman et al., 2007]. Therefore, by normalizing these individuals' fibers (an example of the fibers of subjects 1 to 3 in the MNI space is shown in Fig. 1) and then keeping the fibers that were significant across subjects based on nonparametric one-sample *t*-tests of 5,000 permutations at a level of significance of $P < 0.005$ with family-wise error (FWE) correction, we obtained fibers with the most robust connections with the ROIs across subjects (an example of the results is shown in Fig. 1), and the fiber measurements were then forwarded for statistical analysis of the differences between the three groups.

Hypothalamus analysis

In addition, a hypothalamus mask was generated based on an earlier study of the hypothalamus atlas [Baroncini, et al., 2012] and was then used for the ROI analysis of GMV. GMV was analyzed by the VBM8 toolbox (<http://dbm.neuro.uni-jena.de/vbm/>) with the DARTEL method. The modulated gray matter images were used to extract the mean value within in the hypothalamus mask to represent the GMV. The GMV was then used in the statistical analysis of the differences between the three groups.

Statistical analysis

All of the analyses shown in Tables I and II were performed using SPSS 24.0 software (SPSS, Chicago, IL). Independent two-sample *t*-tests were applied for statistical analysis of the demographic characteristics, ROIs' cortical volume and FA, and GMV of the hypothalamus. $P < 0.05$ was considered to be a significant discrepancy. In addition, abnormalities in PWS and OB were further investigated for progression with the children's age, gender, and BMI-z.

RESULTS

Demographic Characteristics

There were no age or gender differences between the PWS, OB, and control groups. The weight levels were

TABLE II. Ten ROIs exhibiting co-variation in cortical volume in both the PWS and OB subjects compared with controls

ROI #	Hem	Region(s)	Size (mm ³)	MNI coordinate			Statistics of CV			Relevant White Matter	Statistics of FA		
				<i>x</i>	<i>y</i>	<i>z</i>	<i>F_a</i>	<i>F_b</i>	<i>T_c</i>		<i>T_a</i>	<i>T_b</i>	<i>T_c</i>
1	R	MFG	372	44	15	43	7.3	10.3	0.36	ILF, MFG_WM	3.80	-0.69	-3.82
2	R	SFGdor	313	27	18	42	9.9	7.7	-0.35	SLF, SFGdor_WM	3.04	-0.27	-2.84
3	R	SFGdor, MFG	1433	17	50	31	14.3	17.3	0.41	ATR, SFGdor_WM, MFG_WM	2.30	-0.71	-2.86
4	L	SFGdor, MFG	369	-22	51	25	9.2	11.3	-0.14	ATR, MFG_WM, SFGdor_WM	3.03	-0.49	-3.22
5	R	SOG	750	27	-80	21	10.0	11.4	-1.31	FMA, SOG_WM	1.76	-1.29	-2.60
6	L	ACG	417	-7	37	-1	18.7	17.9	0.00	FMI_L, ACG_WM	2.41	-0.80	-3.05
7	R	FFG, PHG	796	33	-36	-19	14.3	14.9	-0.56	IFOF, FFG_WM, PHG_WM	2.89	-0.75	-3.21
8	L	MTG	292	-57	-25	-19	13.7	10.0	-1.30	ILF, SLF, MTG_WM	2.78	-0.95	-3.59
9	L	MTG, ITG	258	-59	-44	-19	8.7	8.3	-0.95	SLF, MTG_WM, ITG_WM	0.62	-0.94	-1.53
10	R	ITG	397	52	-14	-39	10.6	12.1	-0.60	ATR, ITG_WM	3.95	-0.10	-3.56

F: *F* value of the ANOVA results in vertex-wise analysis.

T: *T* value of the two-sample *t*-test results in ROI analysis.

A: Comparison between the PWS and control groups (control - PWS).

B: Comparison between the OB and control groups (control - OB).

C: Comparison between the OB and PWS groups (PWS - OB).

Abbreviations: CV, cortical volume; MFG, middle frontal gyrus; SFGdor, superior frontal gyrus (dorsal); SOG, superior occipital gyrus; ACG, anterior cingulate gyrus; FFG, fusiform gyrus; PHG, parahippocampal gyrus; MTG, middle temporal gyrus; ITG, inferior temporal gyrus; IFL, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus; ART, anterior thalamic radiation; FMA, forceps major; FMI, forceps minor; and IFOF, inferior fronto-occipital fasciculus.

significantly different among the three groups; the OB group had a greater weight level than the PWS and control groups ($P < 0.05$), and the PWS group had a greater weight level than the control group ($P < 0.05$), as illustrated in Table I.

Cortical Volume

Compared with the control group, the PWS and OB groups had a smaller cortical volume ($P < 0.05$, uncorrected), and the results from the PWS versus control and OB versus control groups are shown in Figure 2a,b. The red color indicates regions with a decreased cortical volume in both PWS versus controls and OB versus controls, which were labeled with the AAL atlas. The yellow and blue colors indicate other smaller cortical volumes in the PWS and OB groups, respectively. Although many regions did not overlap, the vertex number of overlapping regions accounts for 37% of the PWS versus control results and 38% of the OB versus control results. Based on the symptoms in PWS and OB, we considered that the overlapping regions played a critical role in food intake regulation. These overlapping regions, which co-varied in the PWS and OB groups, were defined as ROIs (see Table II). ROIs were located in the bilateral dorso-lateral and medial prefrontal cortices (DLPFC and mPFC), right anterior cingulate cortex (ACC), bilateral temporal lobe (including the middle and inferior temporal lobe and fusiform), right occipital lobe, and parahippocampal gyri. In

addition, among these ROIs, the left DLPFC/mPFC and left ACC could not survive after multiple comparison correction, with $CWP > 0.05$.

Interestingly, no differences in cortical volume were found between the PWS and OB groups in these ROIs, with the ROI analysis based on the two-sample *t*-test, as illustrated in Table II and Figure 3, although the PWS group should have more serious impairments. Based on an earlier study [Lindgren et al., 2000], PWS patients exhibit more extreme overeating and have a more serious food-intake disorder than OB subjects. Meanwhile, the differences between PWS and OB (PWS vs. OB) are shown in Figure 2d. No differences were found between the PWS and OB groups, with the exceptions of the right IOG and right MOG ($CWP < 0.05$).

Considering the minimal differences in gray matter observed between PWS and OB subjects, PWS subjects might have distinct white matter lesions that are separate from gray matter lesions.

White Matter Integrity

Therefore, the ROIs were used as seeds for tractography analysis to identify the white matter fibers that were connected to the ROIs. The fibers that were connected to the ROIs are demonstrated in Figure 4 and are labeled with numbers that correspond to the ROIs presented in Table II. These fibers

Figure 2.

Similarities and differences between PWS and OB. Both PWS and OB subjects exhibited smaller cortical volumes than controls ($P < 0.05$, uncorrected). The results overlap in (a) the left hemisphere and (b) right hemisphere. The red color indicates regions with decreased cortical volume in both PWS versus controls and OB versus controls, which were labeled with the AAL atlas. The yellow and blue colors indicate other smaller cortical volumes in the PWS and OB groups, respectively. (c) The vertex number of the overlapping regions account for 37% of PWS versus controls results and 38% of OB versus control results, indicating PWS and OB might share some similar brain dysfunction. (d) No differences were found between the PWS and OB groups, except in the right IOG and right MOG (CWP < 0.05). [Color figure can be viewed at wileyonlinelibrary.com]

robustly existed across the three groups at a level of significance of $P < 0.005$ at the voxel level (corrected) based on non-parametric one-sample t -tests; therefore, the measurements of the white matter fibers were suitable for comparison at the in-group level.

The FA along these fibers was compared between the three groups, and the statistical results are presented in Table II and Figure 5. Compared with the controls, PWS subjects exhibited reduced FA of the white matter fibers that were seeded from 9 out of the 10 ROIs; the 9th ROI, which was located in the middle and inferior temporal gyri, did not have a significantly reduced FA, with $P > 0.05$. The FA reduction in the nine ROIs was consistently found in the PWS group compared with the OB. Additionally, no difference was found between the OB and control subjects. These results validated our hypothesis that the PWS subjects suffered more serious brain structural impairments than the OB subjects.

GMV of Hypothalamus

The statistics results of the GMV for the PWS, OB and control groups are shown in Table III. Compared with the control group, the PWS group had a significantly decreased GMV in the hypothalamus ($P < 0.001$), while the OB group had a slightly decreased GMV in the hypothalamus ($P = 0.05$). These divergences in the PWS and OB hypothalamus further confirmed our hypothesis.

Progression with Age, Gender, and BMI-z

We explored the changes in PWS and OB with age. First, no correlation was found between the cortical volume and age within or across groups, regardless of whether age was controlled in PWS versus controls and OB versus controls. A possible reason for the lack of correlation is that the cortical volume did not significantly change across ages in children and adolescents, as reported in a previous study [Giedd, et al., 1999]. However, FA in ROI-related white matter fibers was found to be significantly correlated with age in the OB and control groups (total $N = 36$), which was consistent with an earlier study [Barnea-Goraly et al., 2005]. Therefore, we built a linear regression model between age and FA across OB and control groups. The absolute residual from PWS' FA to the age-FA linear model was used to estimate the PWS impairment level for corresponding age. We divided PWS into two groups, a younger group (age $<$ median) and older group (age $>$ median). The two-sample t -test was used to examine the difference between the two groups. The results are shown in Table IV. Older PWS subjects had significantly smaller absolute residuals than younger PWS subjects in six ROIs, indicating that older PWS subjects might be less affected than younger patients in regard to these fibers.

In addition, we explored the effect of gender in our MRI findings. However, there was no significant difference between boys and girls.

Figure 3.

Cortical volume between groups. Both the PWS and OB groups exhibited significantly decreased cortical volume in 10 ROIs compared with the control group (ROIs surviving after multiple comparison correction with $CWP < 0.05$ were labeled with *), but no differences were found between the PWS and OB groups across these ROIs ($P > 0.05$).

ROIs' cortical volume were forwarded for Pearson's correlation analysis with subjects' BMI-z across the three groups. Results are shown in Table IV. Cortical volume in five ROIs had significantly negative correlations with BMI-z. However, we could not found significant correlation within each group, due to the small sample size.

DISCUSSION

In the current study, we used T1-weighted MRI and DTI to investigate the brain structural abnormalities associated with PWS and OB. Our results revealed 10 co-varying brain regions with decreased cortical volume in PWS and OB subjects compared with controls. Additionally, we

Figure 4.

White matter fibers connected to ROIs. White matter fiber seeding from 10 ROIs was acquired by tractographic statistics that were applied across all subjects ($P < 0.005$ with FWE correction), which made the measurements of the white matter fibers comparable at the in-group level. The edges of the ROIs are labeled with red lines. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 5.

White matter fibers' FA between groups. FA along the fibers that were seeded from 9 out of the 10 ROIs were significantly reduced in the PWS group (labeled with *, $P < 0.05$ in ROI analysis) compared with the control group. The same results were consistently found between the PWS and OB groups. Additionally, no differences were found between the OB and control subjects.

applied a tractography-based analysis method, and the results confirmed the distinct changes in the white matter fibers connected to co-varying regions in the PWS subjects compared with the controls that were not found in the OB subjects. In addition, PWS subjects had a more serious impairment in the hypothalamus than OB subjects. This study may help us better understand the shared symptoms of OB and PWS that are associated with cognitive-emotional regulation and food intake control as well as the specific symptoms of PWS, which include excessive hyperphagia and constant hunger.

Cortical Volume Decreases in the PWS and OB Groups

OB and PWS subjects exhibited decreased cortical volume in the bilateral frontal lobes, including the DLPFC and MPFC.

The DLPFC is linked to top-down control, especially “executive functions,” goal selection, planning, manipulation of information and response inhibition [Miller and D’Esposito, 2005; Narayanan and Laubach, 2006; Tanji and Hoshi, 2008]. This role in cognitive control could extend to decision-making regarding food intake. Therefore, the impairment in the DLPFC observed in the present study might be suggestive of dysfunctions in the regulation of appetite in PWS and OB subjects. The MPFC is implicated in motivational processing and is involved in the processing of self-referent information [Macrae et al., 2004; Moran et al., 2006]. Early reports noted stronger MPFC activations in individuals with PWS and obesity [Hinton et al., 2006], which are indicative of the role of MPFC in the inhibition of food motivation. The cortical volume decrease in the MPFC in PWS and OB subjects might be associated with increased self-reported disinhibition and hunger, which would contribute to overeating and obesity.

TABLE III. GMV of hypothalamus among PWS, OB, and control groups

ROI	Group			T value			P value		
	Control	PWS	OB	A	B	C	A	B	C
Hypothalamus	0.612 ± 0.114	0.465 ± 0.063	0.547 ± 0.088	4.62	2.02	-2.95	0.00	0.05	0.006

Values are given as the means ± standard deviation.

A: Comparison between the PWS and control groups (control - PWS).

B: Comparison between the OB and control groups (control - OB).

C: Comparison between the PWS and OB groups (PWS - OB).

TABLE IV. MRI findings' progression with age and BMI-z

ROI#	Age ^a		BMI-z ^b	
	<i>T</i>	<i>P</i>	<i>R</i>	<i>P</i>
1	-1.661	0.119	-0.276	0.033 ^c
2	-3.325	0.017 ^c	-0.211	0.081
3	-4.368	0.005 ^c	-0.429	0.001 ^c
4	-5.755	0.001 ^c	-0.192	0.102
5	-2.625	0.039 ^c	-0.144	0.172
6	-5.808	0.001 ^c	-0.19	0.105
7	-1.398	0.157	-0.234	0.06
8	-2.833	0.030 ^c	-0.34	0.011 ^c
9	-6.79	0.000 ^c	-0.314	0.017 ^c
10	-1.424	0.153	-0.311	0.018 ^c

^aTwo-sample *t*-test between younger PWS (age < median) and older PWS (age > median) in ROI-related white matter fibers' FA-decreased level (older - younger). FA-decreased level for corresponding age was represented by the absolute residual from PWS' FA to the age-FA linear model that was built across OB and control subjects.

^bPearson's correlation analysis between ROIs' cortical volume and subjects' BMI-z across the three groups.

^cSignificance with *P* < 0.05.

OB and PWS subjects also exhibited a decreased cortical volume in the right ACC. The ACC is involved in the executive control of internal and external stimuli and regulation of context-dependent behaviors [Devinsky et al., 1995; Fan et al., 2005; Lane et al., 1998]. Impairments of the ACC could contribute to an increased risk of overeating through an imbalance between cognitive and emotional processing.

OB and PWS subjects also exhibited a decreased cortical volume in the bilateral temporal lobe (including MTG, ITG, and FFG). The temporal lobe is related to the processing of sensory input into derived meanings for the appropriate retention of visual memory, language comprehension, and emotion association [Olson et al., 2007]. Although the associations between the temporal lobe and eating disorders need to be clarified, the volume of the temporal lobe exhibits a significant negative correlation with BMI, as reported in a study of 1,428 adults [Taki et al., 2008] and a 24-year longitudinal study [Gustafson et al., 2004]. Our findings are consistent with these large sample studies, suggesting that the dysfunction of the temporal lobe might lead to incorrect regulation of food simulation and emotional appeal.

OB and PWS subjects exhibited a decreased cortical volume in the right SOG compared with controls. In addition, PWS also decreased the cortical volume in the right MOG and IOG compared with OB. The occipital lobe is associated with visual processing of food, and a previous fMRI study of food versus nonfood-related visual stimuli during different states of hunger confirmed that the right SOG plays a crucial role in the coordination of appetite behaviors [Führer et al., 2008]. Our findings might explain the different levels of eating disorders in OB and PWS subjects.

FA Decreases in the PWS Group

Compared to OB and control subjects, PWS subjects exhibited decreased FA along the fibers connected to the ROIs, and similar findings were reported in an early PWS study [Yamada et al., 2006]. Yamada et al. found that FA significantly decreased bilaterally along the posterior limb of the internal capsule, right frontal white matter and splenium of the corpus callosum in PWS [Yamada et al., 2006].

During the coordination of complex cognitions, such as food intake regulation, information needs to be quickly transferred between different brain regions [Morris, 2004], and damage to any part of the white matter connections between these regions could lead to changes in performance [Mesulam, 2000]. Therefore, mild white matter integrity degradation would have a significant effect this coordination [Morris, 2004]. Our study revealed that both gray matter and white matter damage were present in the brain regions associated with food intake in PWS subjects, which explains the specific symptoms of eating disorders in PWS subjects, including excessive hyperphagia and constant hunger.

No FA Reduction in the OB Group

Interestingly, no differences in the white matter integrity were found between OB and control subjects. Unlike adults, whose BMIs are significantly negatively correlated with FA, previous studies have not reached a consensus about whether obese children exhibit impairments in fiber integrity. Yau et al. [2014] studied normal weight and obese adolescents and found multiple white matter clusters with reduced FA in obese subjects, but one cluster in the right prefrontal region had an elevated FA. However, Alosco et al. [2014] did not find significant associations between BMI and FA values in 6- to 18-year-old children. These inconclusive findings in the literature and our findings of unchanged FA in obese children suggest that the influence of obesity on white matter integrity may be variable, regional, and more sensitive to the childhood maturation process.

Limitations

The present study has several limitations. First, PWS is a rare disease, and our small sample size limits the generalization of our findings. Because of the low disease prevalence, it is difficult to generate a large sample size of participants, particularly for MRI studies, which require strict inclusion criteria. Additionally, although we balanced the genders and ages, there were still relatively large differences between the groups. However, small sample sizes and large age ranges are generally acceptable in neuroimaging studies involving PWS [Holsen et al., 2012; Honea et al., 2012; Ogura et al., 2011]. In addition, two ROIs (left DLPFC/mPFC and left ACC) could not survive after multiple comparison correction, which might

be a result of the small sample size and large subjects' variation. Another possible reason for lack of significance might be that DLPFC/mPFC and ACC are not only involved in eating regulation but also in various brain networks. Moreover, the weight level of OB and PWS subjects did not match, which might introduce a bias into our findings. However, it should be noted that the degree of obesity in children with PWS is underestimated because of their decreased muscle mass [Goldstone, 2004]. In addition, PWS includes two major genetic subtypes [Butler, 1990]. The influence of the subtype was not investigated because the two genetic subtypes of PWS exhibit no clinical differences, except hypopigmentation [Butler, 1990]. Because we did not collect enough clinical information in the present study, our MRI findings were not associated with degree of hyperphagia and cognitive abilities in PWS and OB. We plan to investigate these aspects in our future study.

CONCLUSIONS

Our study provides anatomical evidence about the neural mechanism underlying the shared symptoms in PWS and OB subjects, which are associated with cognitive-emotional regulation and food intake control, and the specific symptoms of PWS, including excessive hyperphagia and consistent hunger. These results may help us better understand children's abnormal eating behaviors from both neurogenetic and psychiatric disorders.

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