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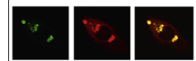
Research Report

The neurobiological drive for overeating implicated in Prader–Willi syndrome

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ABSTRACT

Prader–Willi syndrome (PWS) is a genetic imprinting disorder characterized mainly by hyperphagia and early childhood obesity. Previous fMRI studies examined the activation of eating-related neural circuits in PWS patients with or without exposures to food cues and found an excessive eating motivation and a reduced inhibitory control of cognitive processing of food. However, the effective connectivity between various brain areas or neural circuitry critically implicated in both the biological and behavioral control of overeating in PWS is largely unexplored. The current study combined resting-state fMRI and Granger causality analysis (GCA) techniques to investigate interactive causal influences among key neural pathways underlying overeating in PWS. We first defined the regions of interest (ROIs) that demonstrated significant alterations of the baseline brain activity levels in children with PWS ($n=21$) as compared to that of their normal siblings controls ($n=18$), and then carried out GCA to characterize the region-to-region interactions among these ROIs. Our data revealed significantly enhanced causal influences from the amygdala to the hypothalamus and from both the medial prefrontal cortex and anterior cingulate cortex to the amygdala in patients with PWS ($P<0.001$). These alterations offer new explanations for hypothalamic regulation of homeostatic energy intake and impairment in inhibitory control circuit. The deficits in these dual aspects may jointly contribute

to the extreme hyperphagia in PWS. This study provides both a new methodological and a neurobiological perspective to aid in a better understanding of neural mechanisms underlying obesity in the general public.

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1.   Introduction

Approximately 90 million Americans are overweight or obese and more than 400,000 deaths are related to obesity or its associated diseases per year in the United States (Ogden et al., 2006). While much attention has been given to obesity in the Western world (Gold, 2011), developing countries are not immune to the global obesity epidemic (von Deneen et al., 2011). Causes of obesity are multiple (Dietrich and Horvath, 2009; Liu et al., 2010; Volkow and Wise, 2005; Wang et al., 2009; Zhang et al., 2011) and overconsumption of caloric dense foods is apparently one causal factor in obesity (Holsen et al., 2006; Volkow et al., 2011).

Prader–Willi syndrome (PWS) is a genetic imprinting disorder associated with 15q11–q13 deletion or maternal uniparental disomy of chromosome 15 (Ledbetter et al., 1980; Nicholls et al., 1989). Hyperphagia and obesity are the most notable features of PWS. Individuals with PWS exhibit persistent hunger, food foraging and hoarding; they consume up to three times the normal caloric intake at a meal (Holland et al., 1993). Emerging evidence indicates a hyperresponsive neural network involving brain regions such as hypothalamus, ventromedial prefrontal cortex, insula, and heightened reward circuitry (e.g. nuclear accumbens) 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; Shapira et al., 2005). Our group studied the abnormal brain networks related to eating disorders in children with PWS during the resting-state, and found the alterations of functional connectivity in the default mode network, prefrontal cortex networks, motor sensory network and core network (Zhang et al., 2013). More recently, PWS patients were studied alongside with BMI-matched non-PWS obese subjects by fMRI analysis (Holsen et al., 2012). The study demonstrated hyperactivations in subcortical reward circuitry and hypoactivations in cortical inhibitory regions after eating in the PWS relative to obese individuals. Despite these recent progresses, the neural mechanisms remain unclear from the perspective of interactions between various neural circuitries underlying the extreme hyperphagia in PWS, especially in the non-food-stimulated basal state. PWS serves as an extreme model of hyperphagia of genetic-origin in this regard, and identifying new neural mechanisms driving hyperphagia in PWS may aid in a better understanding of the neurological basis of overconsumption and associated common obesity in the general population.

Previous fMRI studies of acute brain response to high or low caloric food cues in PWS identified the amygdala as a pivotal neuro-locus for cue-enhanced eating (Arana et al., 2003) and

for appetite activation and maintenance (Gottfried et al., 2003; Hinton et al., 2006; Killgore et al., 2003; Kringelbach et al., 2003). The brain regions consisting of the basolateral amygdala (BLA), medial frontal cortex (MPFC) and lateral hypothalamic area (LHA) form a critical neural network in the regulation of eating by learned, motivational cues (DeFalco et al., 2001; Petrovich et al., 2001). In this context, the amygdala nuclei seed a complex network of topographically organized direct and indirect projections to the hypothalamic circuits (Petrovich and Gallagher, 2003) with the BLA and LHA as the essential components of such a system through which learned cues can override satiety and increase eating (Petrovich and Gallagher, 2003, 2007; Petrovich, 2011). The hypothalamic regulation of food intake relies on the reward and motivational neural circuitry to modify eating behavior (Farooqi et al., 2007; Passamonti et al., 2009) and is hence influenced by the frontal cortex including MPFC and ACC that have been implicated in motivational and emotional processing (Martin et al., 2010). Food motivation in non-PWS obese individuals is associated with increased self-reported disinhibition and hunger, and increased activation in the ACC and MPFC (Martin et al., 2010). Dysfunction in the neural circuitry of emotional self-regulation underscores in part the failure to regulate emotions and impulsive aggression (Guroglu et al., 2008). Findings from neuroimaging studies of PWS convey a similar notion in this regard, i.e., there is increased food reward as well as increased disinhibition of cognitive processing of food intake that may jointly contribute to the deficits in executive control of eating behavior (Moran and Westerterp-Plantenga, 2012).

PWS patients exhibit both excessive food motivation and hyperphagia consistently even in the absence of food-related visual stimulation (Cataletto et al., 2011). This implies that the patients with PWS are associated with not only cue-induced abnormal brain responses, but also alterations of the baseline brain activity level that lead to the overeating behavior. While earlier studies have centered on the particular brain areas or neural circuits critical for mediating the hyperphagia in PWS, our current study offers the first of its kind to focus on examining the neural interactions of these brain regions and circuitry that drive overeating in the absence of conditioned cues. We hypothesize that there are abnormalities in the manner by which amygdala drives the hypothalamus and the MPFC and ACC relate to the amygdala in individuals with PWS under a baseline condition devoid of food related stimulation. To test our hypothesis, we employed resting-state fMRI (RS-fMRI) to elucidate differences in the basal brain activity between the PWS children and their healthy sibling controls. Granger causality analysis (GCA), which is an effective connectivity analysis method to characterize the causal influence between different brain

regions, was then used to investigate the causal interactions of the brain areas involved in the drive for overeating.

2. Results

2.1. Altered resting-state ALFF in PWS

Compared with the controls, the PWS patients exhibited significant increased ALFF in the ACC (BA 32/24), left AMY and hypothalamus (Hy), and decreased ALFF in the MPFC (BA 9/10) and right AMY ($P < 0.05$, $n = 21$, FDR correct), and these brain areas were chosen as the ROIs because they are the major components of a specific neural circuitry for controlling and regulating eating (Table 1 and Fig. 1) (Dimitropoulos and Schultz, 2008; Hinton et al., 2006; Holsen et al., 2006; Miller et al., 2007; Shapira et al., 2005). The causal influences among these ROIs were analyzed using the GCA.

Further quantitative analyses by the mean ALFF values were performed to compare the baseline brain activity changes in these ROIs using two sample t-test (Fig. 2). Our results showed a trend of higher ALFF values in the ACC, left amygdala (lAMY), and hypothalamus and lower ALFF values in the MPFC and right amygdala (rAMY) in the PWS group relative to the controls.

2.2. Altered resting-state effective connectivity in PWS

Pair-wise Granger causality analysis was employed to show the alterations of causal influence from one brain region to the other. We found that, compared with the controls, the PWS patients had increased Granger causality in the directions from both the lAMY and rAMY to hypothalamus, from ACC to rAMY, from MPFC to both lAMY and rAMY, and from ACC to MPFC (Fig. 3A).

We then used the normalized ratios $R_{x->y}$ to show the directionality and strength of the effective connectivity among these ROIs (Fig. 3B). Prominent changes in both the directionality and strength of the effective connectivity between the MPFC and rAMY, lAMY and hypothalamus, ACC and rAMY, and between the ACC and MPFC were

associated with the PWS group as compared to the controls, respectively (Fig. 3B). Although the directionality from the rAMY to the hypothalamus remained the same (Fig. 3A), the strength ratio increased dramatically from 3.09 to 18.1,
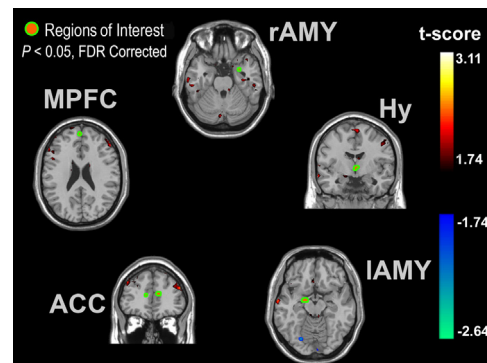

Fig. 1 – The functional mapping of the brain areas demonstrating significant ALFF alterations between the PWS and control groups during resting state ($P < 0.05$, FDR corrected).
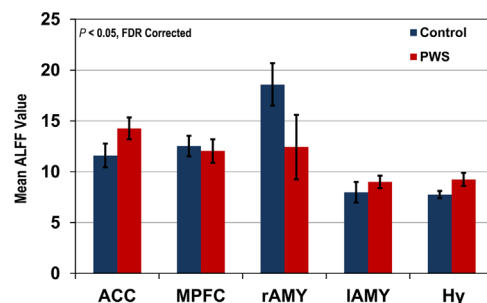

Fig. 2 – Comparison of the mean ALFF values in each ROI between the PWS and control groups. The mean ALFF values were calculated by averaging cross-subjects for the PWS ($n = 21$, red bar) and control ($n = 18$, blue bar) groups. Error bars denoted the standard deviations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1 – Brain regions of interest (ROIs) showing the significant ALFF alterations between the PWS patients and controls by two sample t-tests ($P < 0.05$, FDR corrected).

Distinctions in resting-state brain activity levels between PWS and control

ROIs	Hem	BA	MNI			t Value	Voxel no.
			x	y	z		
MPFC	L	9	−3	−24	63	3.07	170
	R	9/10	6	54	42	2.91	170
ACC	L	32/24	−9	39	15	2.86	675
	R	32	6	9	24	3.11	907
AMY	L		−17	−4	−14	1.98	54
	R		29	2	−25	2.14	189
Hy	L		−3	−6	−12	1.74	72
	R		3	−3	−9	1.99	137

Abbreviation: Hem, hemisphere; BA, Brodmann area; MNI, Montreal Neurological Institute; MPFC, medial prefrontal cortex; ACC, anterior cingulate cortex; AMY, amygdala; Hy, hypothalamus.
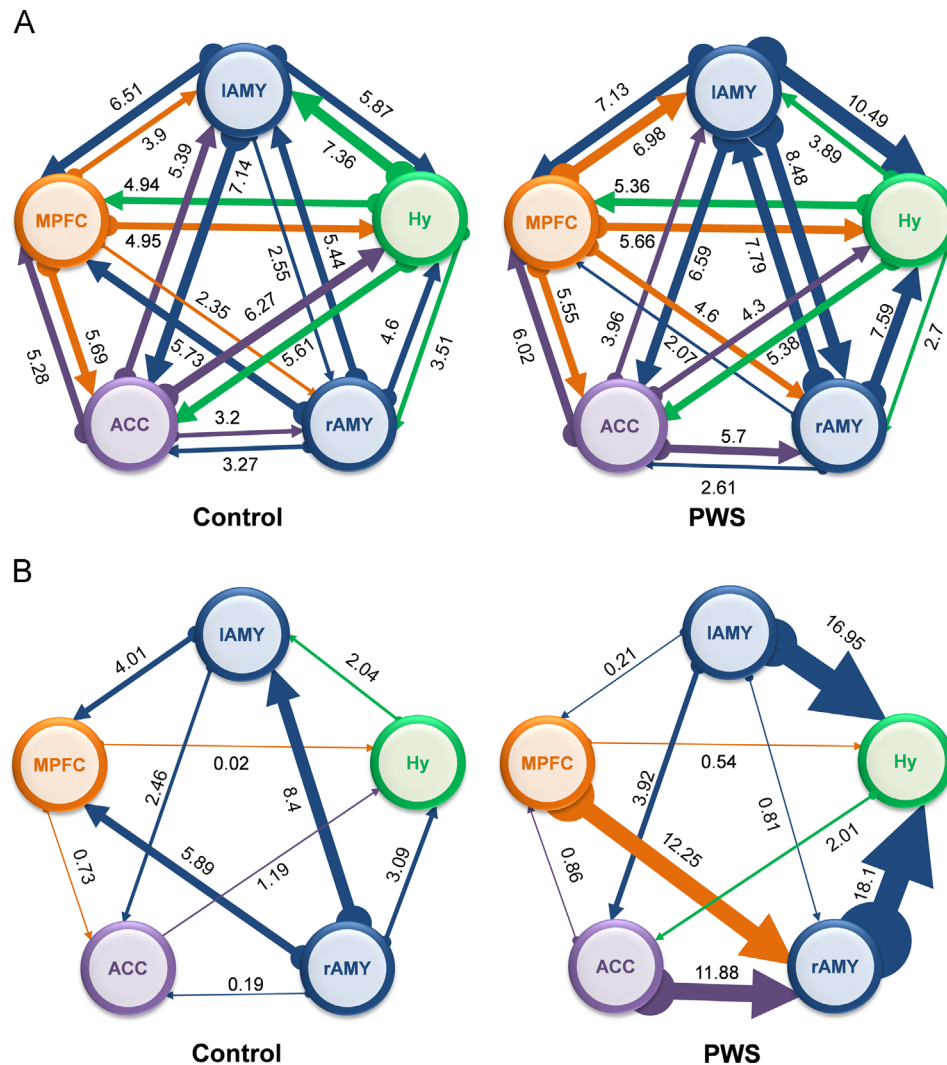
Fig. 3 – Granger causal influences between select pair–wise ROIs demonstrating the directional driving effects from one brain region to the other. (A) Granger causality (GC) values normalized to a standard scale were displayed and compared. The strength of the pair-wise ROIs showed increased Granger causal influence from both the lAMY and rAMY to the hypothalamus, from the ACC to the rAMY, from the MPFC to both the lAMY and rAMY, and from the ACC to the MPFC in the PWS patients. (B) Comparisons of the normalized ratios of GC values among the ROIs. The pair-wise MPFC and rAMY, lAMY and hypothalamus, ACC and rAMY, as well as ACC and MPFC revealed alterations in both directionality and strength of the effective connectivity in the PWS patients relative to the controls.

showing a significant enhancement in their causal relationship (Fig. 3B).

3. Discussion

In the current study, we adopted a unique approach to explore new neural mechanisms underlying the extreme hyperphagia in PWS children in comparison with their healthy siblings. Specifically, we combined the RS-fMRI examination of ROIs demonstrating significant alterations of baseline brain activity levels in the patients with a subsequent GCA of the interactive causal influences between the different ROIs. This novel methodological combination produced salient findings in the following two aspects: (1) under the basal condition, the PWS patients exhibited altered ALFF

values in the brain regions including the MPFC, ACC, amygdala and hypothalamus; (2) as compared to the control group, the pair-wised connections such as MPFC and rAMY, lAMY and hypothalamus, ACC and rAMY as well as ACC and MPFC showed alterations in both directionality and strength of the effective connectivity, respectively, in the PWS group.

3.1. Aberrant baseline brain activities in PWS

The RS-fMRI, or the ALFF in particular, is an effective index reflecting baseline brain activities of a regulatory neural network in the default mode (Jiao et al., 2011a). Our observation of significant ALFF changes in the MPFC, ACC, amygdala and hypothalamus with PWS (Figs. 1 and 2) is consistent with the result of abnormal neural circuitry activation in similar brain regions from previous PWS neuroimaging

studies under food-stimulation conditions. (Dimitropoulos and Schultz, 2008; Hinton et al., 2006; Holsen et al., 2006; Miller et al., 2007). An earlier PET study noted an increase in the basal metabolism in the amygdala in PWS, suggestive of a role of amygdala in the cognitive aspect of hyperphagia involving the drive and anxiety at the baseline level (Kim et al., 2006). Our findings are in support of such a notion.

3.2.   Driving force from the amygdala to hypothalamus

One pair of the ROIs showing significant increase in Granger causality influence is from the amygdala to the hypothalamus. In fact, the causal influence from the rAMY to the hypothalamus was significantly higher (7.09 vs. 4.6) and the causal influence from the lAMY to the hypothalamus was also significantly higher (10.49 vs. 5.87) in PWS compared to sibling controls (Fig. 3A). These substantially enhanced causal influences from amygdala layered onto the hypothalamus may mar the ability of the latter to exert its regulatory restraint on excessive eating behavior beyond the normal caloric and nutritional need, eventually leading to hyperphagia.

Early studies indicated an overactivity of the hypothalamus to food cues (Dimitropoulos and Schultz, 2008) and a delayed signal reduction after glucose loading in the hypothalamus, ventromedial PFC, and NAc (Shapira et al., 2005). Although such evidence apparently pinpoints a central role of the hypothalamus in the control of overeating behavior in PWS, the effective connectivity between the hypothalamus and amygdala, and other potential relevant brain sites remains unclear. Our study intends to fill the gap in this knowledge. It is known that amygdala nuclei seed a complex neural network of topographically organized direct and indirect projections to the hypothalamus circuits (Petrovich et al., 2001). The BLA, for example, is implicated in learning and projects substantially to the LHA (Petrovich et al., 2001). Both rodents (McDonald and White, 1993) and humans (Johnsrude et al., 2000) with damaged amygdala are impaired in the conditioned cue preference, and destroying the connectivity between the two structures by a physical lesion (Petrovich and Gallagher, 2003) signifies the BLA and LHA as critical components of a neural system through which learned cues override satiety and increase eating. Greater activation in the rAMY in PWS compared to the control samples in response to high-calorie foods as opposed to non-food objects has been reported (Dimitropoulos and Schultz, 2008). Our data provides a new perspective on describing the functional relation between the amygdala and hypothalamus during the resting-state devoid of food cues in PWS. We contend that the aberrant causal influence from the amygdala to hypothalamus is persistent and intrinsic to the patients and that it produces anomalous reinforcing properties and motivational salience with or without food stimulation, which may ultimately override the normal regulation of appetite and drive PWS individuals to overeat.

3.3.   Driving force from the MPFC and ACC to the rAMY

Abnormal driving effects were also found from the MPFC and ACC to the rAMY. Both the causal influence from the MPFC to the rAMY (4.60 vs. 2.35) and from the ACC to the rAMY (5.70 vs. 3.20) were significantly higher in PWS compared to controls (Fig. 3A). Moreover, the strength and directionality alterations (Fig. 3B) of the region-to-region causal influence revealed a dysfunctional inhibitory control that persisted in the absence of food cues in PWS patients.

Food motivation in obese individuals is associated with increased self-reported disinhibition and food reward involving both the ACC and MPFC in motivational processing (Martin et al., 2010). Early work demonstrated enhanced MPFC activation in individuals with PWS, but failed to show an association between this activation and ratings of the reward value of various foods these individuals (Hinton et al., 2006). Our study showing activation of MPFC in the resting state may explain the persistent thinking about food that people with PWS report. In regards to the ACC region, it is indicated in the executive control of the internal and external stimuli-related, context-dependent behaviors involving evaluation of the salience of emotional information and modulation of emotional response (Bush et al., 2000; Devinsky et al., 1995; Lane et al., 1998). The ACC may contribute to an imbalance between cognitive and emotional processing and consequentially an increased risk to overeat (Kullmann et al., 2012). In the present study, our GCA analysis uncovered abnormally enhanced driving forces from both the MPFC and ACC to amygdala, which are consistent with the observations in patients with PWS of an impaired inhibition function in response to food cues.

Despite our novel experimental approach and salient findings, the present study has its limitations. Due to the low prevalence of the disease, it is difficult to recruit a large number of participants especially for conducting an fMRI study which requires strict inclusion criteria. The small sample size limits the generalization of our observations. Additionally, even though we balanced the gender and age range among the PWS patients, the control samples size, is relatively larger in comparison. Although we performed the regression analysis on the effect of IQ confound, its impact could not be completely removed. Whether or not the Granger causality analysis is actually capable of characterizing directionality information with BOLD-fMRI data is still under debate. Due to the size of the structures relative to the resolution of the acquisition and inadequate alignment, the BOLD imaging of structures in the brainstem is not reliable.

4.   Conclusion

In the present study, we employed the RS-fMRI in conjunction with the GCA to elucidate the differences of baseline brain activity levels and the interactive causal influences of the major brain regions related to hyperphagia in children with PWS versus their healthy siblings. Our data demonstrate unusual driving forces from the amygdala to the hypothalamus and from the MPFC and ACC to the amygdala in PWS. These findings implicate that the long-term effect of the aberrant learned cues generated from amygdala may override the normal function of the hypothalamus, resulting in deregulation of homeostatic energy intake and dysfunction of inhibitory control. Furthermore, the alterations of the

Table 2 – Demographic information of the PWS patients and sibling controls.			
	PWS ($N=21$) (mean$\pm$SD)	Sibling control ($N=18$) (mean$\pm$SD)	P Value
Age (yrs)	9.3 ± 7.3	11.1 ± 8.4	0.752
Gender	11M/10F	8M/10F	0.632
BMI	33.1 ± 1.0	24.8 ± 2.7	0.020
IQ	64.7 ± 11.7	103.8 ± 11.6	< 0.05
Genetic abnormality	Deletion of the chromosomal 15q11–13	N/A	N/A

Abbreviation: PWS, Prader–Willi syndrome; BMI, body mass index.

interactive causal influences within the hypothalamic neural circuitry may link to appetite and eating behavior in patients with PWS. Obesity research in general may benefit from our unique study design and new knowledge of neural mechanisms for gaining more insight into the neurological basis of overeating and associated dietary obesity in the general population.

5. Experimental procedure

5.1. Participants

A total of 27 patients with PWS and 21 of their siblings participated in the study. The fMRI scans will detect both brain functional changes and neuroanatomical variability of these subjects (Miller et al., 2007). Among them, 21 children with PWS (10 female, 11 male; mean age: 9.3 ± 7.3 yrs), and 18 healthy siblings of the PWS patients consisting of a control group (10 female, 8 male; mean age: 11.1 ± 8.4 yrs) were selected (Table 2). Other participants were removed from the current analysis due to either excessive head motion (translation more than 3 mm and rotation more than 3 degrees) or failure to perform the functional scanning. The PWS subjects were significantly more obese than controls, as assessed by body mass index (BMI, 33.1 vs. 24.8; $P=0.02$) (Table 2). None of the PWS patients were being treated with growth hormone or estrogen/androgen replacement at the time of MRI scanning. Molecular testing was performed on all PWS subjects, all of them had a deletion of the chromosomal 15q11–13 region. The overall research protocol was approved by the Institutional Review Board at the University of Florida and informed consent was obtained from each participant or from his/her legal guardian.

5.2. MRI acquisition

The experiments were carried out on a 3.0 T head-dedicated Siemens Allegra MRI scanner. Before the fMRI scan, all of the subjects were fasting for average 3 h. A set of T1-weighted high-resolution structural images were acquired using an MPRAGE sequence with matrix size$=512\times512$, TR$=1500$ ms, TE$=4.38$ ms, FOV$=240\times240$ mm^2, flip angle$=8$ degree and 160 continuous axial slices. Then, a gradient echo T2*-weighted EPI sequence was used for acquiring resting state functional images with the following parameter: TR$=3000$ ms, TE$=25$ ms, flip angle$=90$ degree; matrix size$=64\times64$, FOV$=240\times240$ mm^2, in-plane resolution of 3.75×3.75 mm^2, 36 axial slices. The scan for RS-fMRI lasted for 300 s,

containing 100 brain volumes. Participants were placed in the scanner in a supine position using a foam head holder to lessen motion. Earplugs were used to reduce scanner noise.

5.3. Image processing

(1) All the functional images first underwent slice-time correction (i.e., sinc interpolation to temporally align each slice to the start of each volume), and were then realigned to correct interscan head movement. (2) Framewise displacement (FD) was calculated to index the head movement from one volume to the next (Power et al., 2012). (3) Realigned images were normalized to the standard EPI template and resampled to a voxel size of $3\times3\times3$ mm^3. (4) Demeaning and detrending were performed and head motion parameters, white matter signals and cerebrospinal fluid signals were regressed out as nuisance covariates (Power et al., 2014). (5) Root mean squared signal change (DVARS) which indicates the change in signal intensity from one volume to the next, and standard deviation (SD) were respectively calculated (Power et al., 2014). (6) We compared the three parameters (i.e., FD, DVARS and SD) of each subject to the criteria proposed by Power and his colleague (Power et al., 2012). (7) If any subject did not meet the requirements (FD value less than 0.5, and ΔBOLD of DVARS less than 0.5%) (Power et al., 2012), we formed a temporal mask using the SD obtained from quality measures, and performed a least-squares spectral decomposition of the 'good' data (meet the requirements) and this decomposition was used to reconstitute data at 'bad' timepoints (did not meet the requirements) (Mathias et al., 2004; Power et al., 2014). Thus, the 'good' data defined the frequency characteristics of signals that then replace the 'bad' data (Power et al., 2014). (8) A band-pass filter (0.01–0.08 Hz) was employed to remove the effects of very low frequency drift and high frequency noise using the REST toolkit (http://resting-fmri.sourceforge.net). (9) Finally, the functional images were spatially smoothed with a Gaussian kernel of $6\times6\times6$ mm^3 Full-Width Half-Maximum to decrease spatial noise.

5.4. ROI analysis

Slow fluctuations of brain activity are a fundamental feature of the resting brain, and their presence is vital to determine correlated activity between brain regions and define resting state networks. The relative magnitude of these fluctuations can differ between brain regions and between subjects, and thus may act as a marker of individual differences or dysfunction. Amplitude of Low Frequency Fluctuations (ALFF)

([Zang et al., 2007](#)) is a neuroimaging method that can be used to measure the spontaneous fluctuations in BOLD-fMRI signal intensity, and it has been investigated as part of a reliable biomarker for many neurological conditions such as ADHD ([Zang et al., 2007](#)), schizophrenia ([Zhou et al., 2014](#)), Alzheimer's ([Weiler et al., 2014](#)) and Parkinson's ([Yao et al., 2015](#)) diseases, anxiety ([Zhang et al., 2014](#)) and major depressive disorder ([Wei et al., 2014](#); [Liu et al., 2014](#)). The ALFF analysis was therefore carried out using the REST software to define the ROIs. The calculation procedure was the same as that reported in the previous study ([Zang et al., 2007](#)). The filtered time series of each voxel was transformed to the frequency domain with a fast Fourier transform and the power spectrum was calculated. Because the power of a given frequency is proportional to the square of the amplitude of this frequency component, the square root was calculated at each frequency of the power spectrum and the averaged square root was obtained across 0.01–0.08 Hz at each voxel ([Zang et al., 2007](#)). This averaged square root was taken as the ALFF measurement. For standardization, the ALFF of each voxel was further divided by the global mean ALFF values ([Zang et al., 2007](#)). After we obtained the ALFF value, the voxel-wise two-sample t-tests were employed to compare the differences in ALFF between the PWS and the control group (PWS>HC). We then performed regression analysis on the effect of age by considering the significant amount of neural development that occurs in childhood, through adolescence, and into young adulthood. Gender and IQ were also regressed out. The brain regions showing significant ALFF alterations and related to eating disorders were selected as the ROIs ($P<0.05$, FDR corrected) for further Granger causality analysis. The ALFF values of each ROI were then averaged cross subjects in the PWS and control groups, respectively.

5.5. Granger causality analysis

After obtaining the ROIs with significant ALFF differences, GCA ([Ding et al., 2006](#)) was performed among them. Recently, the GCA has been applied more frequently in the analysis of functional neuroimaging data including electroencephalography ([Chen et al., 2006](#)) and fMRI ([Bressler et al., 2008](#); [Jiao et al., 2011b](#); [Liao et al., 2010](#); [Roebroeck et al., 2005](#)). The original GCA technique is based on the concept of predictability; a signal time series $y(t)$ is said to causally influence a signal time series $x(t)$ if the future course of x is more accurately predicted based on the histories of both of the signals x and y compared to signal x alone ([Granger, 1969](#)).

In the current analysis, given any two ROI's time series $x(t)$ and $y(t)$, the following time domain pair-wise GCA components were evaluated based on an order-two VAR model: the causal influence from $x(t)$ to $y(t)$ ($F_{x\to y}$) and the causal influence from $y(t)$ to $x(t)$ ($F_{y\to x}$). Then, the alterations in the strength of effective connectivity between the pair-wise ROIs were calculated by computing the differences in Granger values between the PWS and control groups. Finally, the differences were normalized using the following ratio ([Sridharan et al., 2008](#)):

$$R_{x\to y} = (F_{x\to y} - F_{y\to x})/(F_{y\to x} + F_{y\to x})$$

to show the between-group alterations of the causal influence from one region to the other ([Sridharan et al., 2008](#)).

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