

Abnormal Degree Centrality in the Right Inferior Frontal Gyrus During Urine Holding in Children With Primary Monosymptomatic Nocturnal Enuresis

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Abstract

Background: Primary monosymptomatic nocturnal enuresis (PMNE) is a common disorder among school-age children. Previous research has suggested that the prefrontal cortex (PFC) is essential to maintain urine storage in bladder control. We hypothesized that children with PMNE have functional deficits in several brain regions, especially the PFC, during urine storage. In this work, we investigated 30 children with PMNE and 28 controls in a state of natural urine holding to evaluate dysfunction in the bladder control network by applying degree centrality (DC) analysis methods based on resting-state functional magnetic resonance imaging. And seed-based functional connectivity (FC) analysis was used to investigate whether the dysfunctional areas exhibited altered FC with other brain regions.

Results: Compared with the typical healthy children, the children with PMNE showed increased DC in the right inferior frontal gyrus (IFG). Also, the right IFG showed increased connectivity with the left middle and inferior frontal gyri and the right precuneus extending to the cuneus in the children with PMNE.

Conclusion: The children with PMNE showed abnormal neural activity during urine storage and exhibited increased DC in the right IFG and increased connectivity with the left PFC and right precuneus during urine storage. These results suggest that compensatory effects may be associated with the right IFG combined with the precuneus and left PFC working together to maintain high vigilance and improve micturition's inhibition function to preserve the state of urine holding in children with PMNE.

Introduction

The standardization committee of the International Children's Continence Society and the American Psychiatric Association have defined children aged ≥ 5 years who have intermittent incontinence of urine during sleep, have never previously been dry for less than 6 months, and have no other lower urinary tract symptoms as having primary monosymptomatic nocturnal enuresis (PMNE) [1]. Enuresis is a common complaint in children, with a prevalence of 10% of children at the age of 7 years, 3% at 11–12 years, and 1% at 16–17 years[2]. Approximately 48% of enuretic children had poor school performance, and the quality of life scores of nocturnal enuresis group was significantly lower than that of healthy control group[3]. PMNE is a common disease that has a tremendous psychological impact on afflicted children and their families' economic impact. The pathogenesis of enuresis are suggested to be related to genetic factors, nocturnal detrusor overactivity, nocturnal polyuria, high arousal thresholds, psychological factors, as well as central nervous system dysfunction[4].

Children with enuresis will have involuntary bedwetting at night; the brainstem mainly controls involuntary urination. Some higher brain regions are also involved in urinary control, such as the anterior cingulate cortex prefrontal cortex (PFC), supplementary motor area, and the thalamus[5]. Previous literature has found that the PFC, which is dominated by inferior frontal gyrus (IFG), is the critical area for this inhibition function[6, 7].

Previous research has suggested that the PFC is important for bladder control; the PFC inhibits the midbrain periaqueductal gray matter during the maintenance of the urine storage phase[8, 9]. Children who have poor PFC function could have abnormal urine storage. In a previous paper, we reported that children with PMNE exhibited decreased brain activation in the bilateral inferior frontal gyri, the right superior frontal gyrus, and the middle frontal gyrus during a response inhibition task[10]. Resting-state functional magnetic resonance imaging (fMRI) studies have found that, compared with healthy controls, PMNE children not only exhibited an increased amplitude of low-frequency fluctuation in the medial PFC and regional homogeneity in the medial PFC and precuneus but also brain exhibited network alterations, for example, decreased local and global efficiency of information processing and integration[11, 12]. These previous studies indicated that the PFC displays functional abnormalities in children with PMNE, which would be related to the control of micturition.

Therefore, we hypothesized that children with PMNE have functional deficits in several brain regions, especially the PFC, during urine storage. In this study, we analysed fMRI data in the state of natural urine holding to evaluate dysfunction in the bladder control network in children with PMNE by applying degree centrality (DC) analysis methods. DC is a graph-based measurement of whole-brain functional connections and have widely used in brain research. It was used to investigate the characteristics of intrinsic connectivity from the perspective of the whole network[13]. Jiang et al. have reported that primary nocturnal enuresis children exhibited lower DC value in several brain areas under resting state and might be associated with their impaired attention[14]. Furthermore, we employed functional connectivity (FC) with a seed-based method to investigate whether the dysfunctional brain areas show abnormal FC with specific brain regions.

Results

PMNE children showed increased DC in the right IFG (see figure 1 and table 1 compared with the controls. As an ROI (region of interest), the right IFG showed increased connectivity with the left middle and inferior frontal gyri and right precuneus extending to the cuneus in the children with PMNE compared with the healthy controls (see figure 2 and table 1).

Table 1. Between-group differences in DC and FC

Brain regions	T value	Number of voxels	MNI coordinates		
			x	y	z
DC (PMNE group > healthy group)					
Right inferior frontal gyrus	6.05	57	48	18	3
FC (PMNE group > healthy group)					
Left middle and inferior frontal gyri	4.54	83	-30	42	21
Right precuneus extending to cuneus	4.10	65	6	-78	36

PMNE = primary monosymptomatic nocturnal enuresis; DC = degree centrality; FC = functional connectivity. MNI = Montreal Neurological Institute.

All the statistical results reported reached voxel-level uncorrected $p < 0.001$ and cluster-level $p < 0.05$ with family-wise error correction.

Discussion

We hypothesized that children with PMNE have functional deficits during urine storage. The results in the present study are consistent with our hypotheses. The children with PMNE exhibited increased DC in the right IFG and increased connectivity with the left middle and inferior frontal gyri and right precuneus extending to the cuneus during urine storage. DC analysis can help us to form a functional integration map at the voxel level, and DC values can also be regarded as the functional connection density. An increased DC value indicates an increased number of direct connections, and reflects the increased centrality or importance of specific voxels in the brain network[15].

The IFG is associated with micturition control [16, 17]. Activation of the PFC has been found both in people who wanted to empty but could not as well as those who could successfully void[18]. When comparing the urine storage period urine-withholding state with the resting state, there was a significant increase in blood flow in the bilateral inferior frontal gyri and the right superior and the middle temporal gyri, and the right IFG was distinctly prominent among the regions [19]. Increased blood flow was also found in the right IFG during unsuccessful attempts at micturition[20]. Hruz et al. investigated that emptying the bladder showed increased brain activity in the right IFG compared with the passive filling of the bladder[21]. Micturition versus rest was associated with bilateral activation of areas close to the IFG, postcentral gyrus, and other brain areas[22]. These findings indicated that the decision to void is probably generated in the PFC, especially in the IFG.

In healthy individuals, the inferior, medial and superior frontal gyri have been identified as ROIs in voiding [17]. In previous literatures, PFC is considered to be related to withdrawing decisions in bladder control models[8, 9]. The model showed that PFC prevented voiding by inhibiting periaqueductal gray matter in the midbrain during urine storage [8, 9]. In most cases, the bladder is in the storage stage, and PFC's role is to inhibit the periaqueductal gray matter. When the afferent signal to the periaqueductal gray matter exceeds a certain threshold, the password is sent to the PFC to determine whether voiding is safe and appropriate [23]. Dysfunction in the PFC might induce abnormal voiding behavior. In addition, previous studies have proposed that the right IFG in humans is critical for inhibiting response tendencies[24]. Our previous study also showed that children with PMNE exhibited abnormal activation in the bilateral inferior frontal gyri, the right superior frontal gyrus, and middle frontal gyrus during a response inhibition task [10]. Thus, increased DC in the right IFG in children with PMNE may indicate that the patient tried to enhance response inhibition function and decided not to void to prevent voiding during urine holding. We speculate that IFG may play an essential role in the pathology of PMNE by playing a compensatory effect while holding urine in the daytime and maintaining the relatively normal function of holding urine.

Furthermore, the children with PMNE exhibited increased connectivity between the IFG and the left middle and inferior frontal gyri and the right precuneus extending to the cuneus. The precuneus is located in the posteromedial cortex of the parietal lobe. It has been proposed that the precuneus is the central node of the [default mode network](#) with particular relevance for the maintenance of the conscious state and perhaps the most connected hub in the cortex[25, 26]. The precuneus is involved in episodic memory retrieval, visuospatial imagery, self-processing and consciousness and may participate in the integration of multiple neural information producing a conscious self-percept [26]. We speculate that children with PMNE may have more stress during the period of holding urine, and they are more alert. The children with PMNE exhibited increased connectivity between the IFG and the left middle and inferior frontal gyri and the right precuneus extending to the cuneus, which may indicate that the IFG combined with the precuneus and left PFC to work together to maintain high vigilance and improve the inhibition function of micturition to achieve urine holding during scanning.

While our research revealed that the children with PMNE exhibited an increased DC in the right IFG and increased connectivity with other brain areas during urine storage, our study had several limitations. First, considering that children are in a rapid developmental period and that individual differences were noticeable, the results need to be further verified with larger samples. In addition, in this experiment, the children were willing to drink 300 ml of water to reach the state of bladder filling, which may be closest to the bladder's state in their daily lives. However, there are no quantitative data on bladder filling, so we could not analyse the correlation between activity in the brain and the bladder filling state.

Conclusions

In summary, current research shows that children with PMNE showed abnormal neural activity during urine storage. The children with PMNE exhibited an increased DC in the right IFG and increased connectivity with the left middle and inferior frontal gyri and the right precuneus extending to the cuneus during urine storage. These results indicate that compensatory effects may be related to the right IFG combined with the precuneus and left PFC working together to maintain high vigilance and improve micturition's inhibition function to preserve the state of urine holding in children with PMNE.

Methods

Subjects

Thirty of 7-12 years old children with PMNE were recruited from among outpatients of Shanghai Children's Medical Center. These patients were diagnosed as PMNE by a senior doctor according to the international Classification of diseases and The Diagnostic and Statistical Manual of Mental Disorders. The inclusion criteria were as follows: 1) The patient wetted the bed during the night more than or equal twice a week for three months but did not during the day. 2) Their symptoms were not caused by any related disease or drug, 3) Their symptoms continued for more than six months. Twenty-eight age- and gender-matched healthy controls were recruited if they had not wet their bed after age 5. All subjects were

right-handed and all neurological and psychiatric diseases were excluded based on clinical examination and a structured interview; more details, see Table 2 and additional file 1.

Table 2. General clinical information for the PMNE group and healthy group.

Measures	PMNE group (n = 30)	Healthy group (n = 28)
Age, years	9.34 ± 1.37	9.62 ± 1.45
Gender, male/female	15/15	14/14
Years of education	3.31 ± 1.23	3.22 ± 1.42
Bed-wetting frequency, per week	2.16 ± 0.05	-
Number of patients never waking up for voluntary voiding	15 (n=30)	-

PMNE = primary monosymptomatic nocturnal enuresis.

MRI image acquisition

The MRI data were acquired using a 3.0 Tesla scanner (Siemens Trio Tim, Erlangen, Germany) that utilized a 12 channels head coil. The children were required to drink approximately 300 ml of water, and the functional MRI scan began when they felt like urinating. During the scan, the subjects were instructed to hold urine naturally, remain at rest with close their eyes, not fall asleep, and minimize movement. Head motion was confined to less than 2 mm or 2°.

We used a high-resolution T1-weighted 3-dimensional magnetization-prepared rapid acquisition gradient-echo (MPRAGE) pulse sequence to obtained the whole brain anatomical images. The parameters were as follows: echo time (TE) = 3.42 ms, repetition time (TR) = 1900 ms, inversion time = 900 ms, flip angle = 9°, field of view (FOV) = 240 × 240 mm², matrix size = 256 × 256, slice thickness = 1.0 mm, and 192 sagittal slices. The resting-state functional data were acquired using a T₂^{*}-weighed gradient-echo echo-planar-imaging (EPI) sequence, the parameters of the sequence were as follows: FOV = 220 × 220 mm², matrix size = 64 × 64, TR = 2000 ms, TE = 30 ms, slice thickness = 3 mm, 32 axial slices to cover the whole brain, 210 volumes.

fMRI image analysis

fMRI data preprocessing

Functional images analysed with statistical parametric mapping software (SPM12; <http://www.fil.ion.ucl.ac.uk/spm/>), the Data Processing Assistant for Resting-State fMRI (DPARSF; <http://rfmri.org/DPARSF>), and Matlab (MathWorks, Natick, MA) software on a personal computer. The first ten volumes of functional data were discarded for each participant to avoid MRI system instability

and allow the participant adaptation to the machine noise. The remaining 200 volumes were then corrected for the acquisition time delay using slice-timing, realignment was used to correct for head motion. After these corrections, the series of functional images for each participant were segmented and normalized, which included individual structural and functional images that were coregistered and segmented into gray matter, white matter and cerebrospinal fluid, after that the functional images were normalized to Montreal Neurological Institute stereotaxic space (3-mm isotropic voxels). In this study, the tissue probability template was created according to children's age and gender by the Template-O-Matic toolbox (TOM, <http://141.35.69.218/wordpress/software/tom/>). After that, the images were bandpass filtered (0.01-0.08 Hz) to reduce the influences of low-frequency drift and high-frequency respiratory and cardiac noise. The spatial smoothing was performed on the functional images using a 6-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel.

Each participant's fMRI data were denoised through linear regression of confounding effects including movement estimates (Friston 24-parameter, including current and past position parameters) and blood oxygen level-dependent signals in cerebrospinal fluid and white matter by the CompCor technique, which removed MRI signals derived from the respiratory and cardiac cycles. We did not regress out the global mean timecourse in consideration of its relation to global brain activity, which would be problematic for calculating DC. DC values were based on the correlation between one voxel and the rest of the whole brain, as described later in this article. Framewise displacement (FD) was calculated for each participant and included in the group analysis.

DC analysis

After preprocessing, the normalized and filtered images were used to calculate binarized DC measures using DPARSF. First, Pearson correlations between the time series of all gray voxel pairs were calculated, and a whole-brain FC matrix was constructed for each subject. Then, we obtained an undirected binarized matrix. If the Pearson's coefficient between the two voxels was < 0.25 , the elements of the binarized matrix were set to 0; otherwise, they were set to 1. Then, the voxels that had low temporal correlation due to signal noise were excluded. From the perspective of graph theory, the DC value of each voxel reflects the number of edges connected to the given voxel (vertex), which indicates its central role in transferring information across brain regions. Standardized binarized DC maps were obtained by dividing by the global average of DC values.

Seed-based FC analysis

The brain regions in which the patient group showed significantly different DC from the healthy group were defined as ROIs. In brief, Pearson correlation coefficients of the functional time series were calculated between the ROIs and the whole brain voxel-by-voxel using DPARSF. Individual correlation coefficients were then transformed into z-scores to improve normality and acquire the zFC maps.

Statistical analysis

Individual DC and zFC images entered into the group-level analyses using SPM. Two sample t-tests were conducted to analyse the between-group differences. Each subject's age, gender and FD were as covariates. All the statistical results reported reached voxel-level uncorrected $p < 0.001$ and cluster-level $p < 0.05$ with family-wise error correction.

Abbreviations

PMNE: primary monosymptomatic nocturnal enuresis; FC: functional connectivity; DC: degree centrality; PFC: prefrontal cortex; IFG: inferior frontal gyrus; fMRI: functional magnetic resonance imaging (fMRI); DPARSF: Data Processing Assistant for Resting-State fMRI; TR: repetition time; TE: echo time; FOV: field of view; FD: framewise displacement.

Declarations

Ethics approval and consent to participate

This study was approved by the East China Normal University Committee on Human Research (HR2015/03011), which were conducted in accordance with the guidelines of the Declaration of Helsinki. All the children and their parents signed an informed form approved by the committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XD, JM and MW designed the study. MW, XZ and ZQ acquired the data. MW, and ZQ analysed the data. MW, XZ, JM and XD interpreted the data. XD and MW wrote the main manuscript text. All authors read

and approved the final manuscript.

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Not applicable.

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Figures

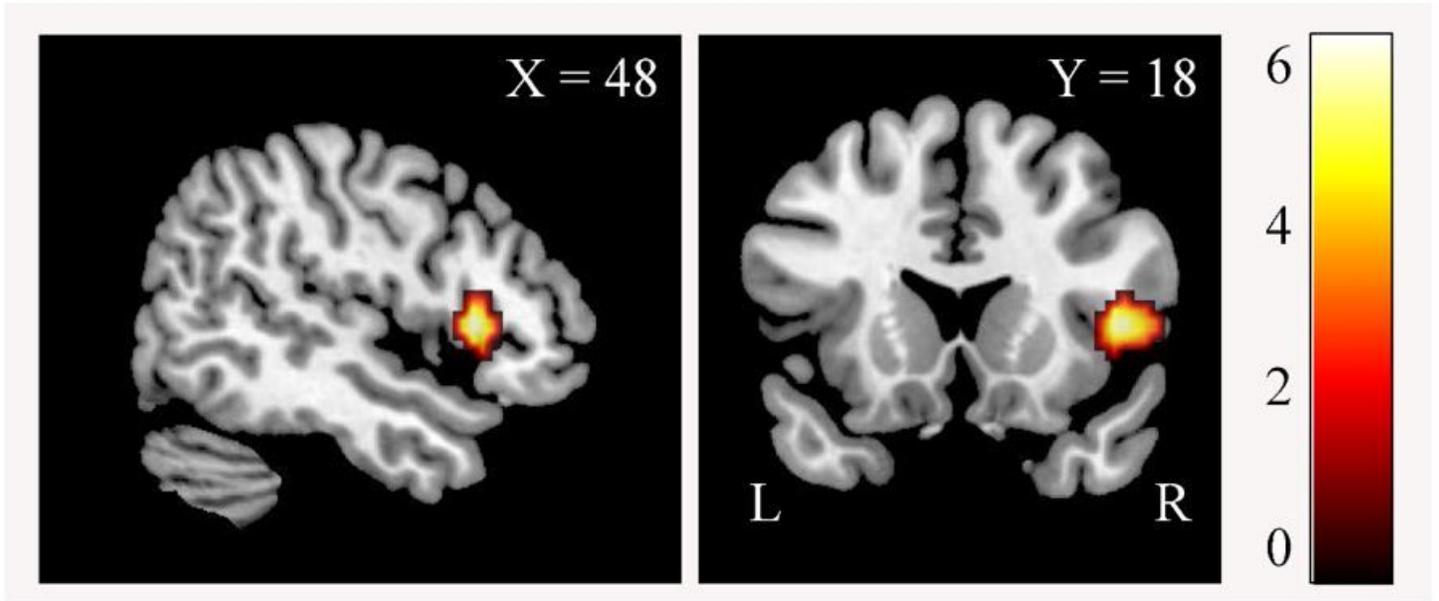


Figure 1

Significant differences in degree centrality between children with PMNE and healthy controls. PMNE = Primary monosymptomatic nocturnal enuresis. PMNE group showed increased DC in the right inferior frontal gyrus.

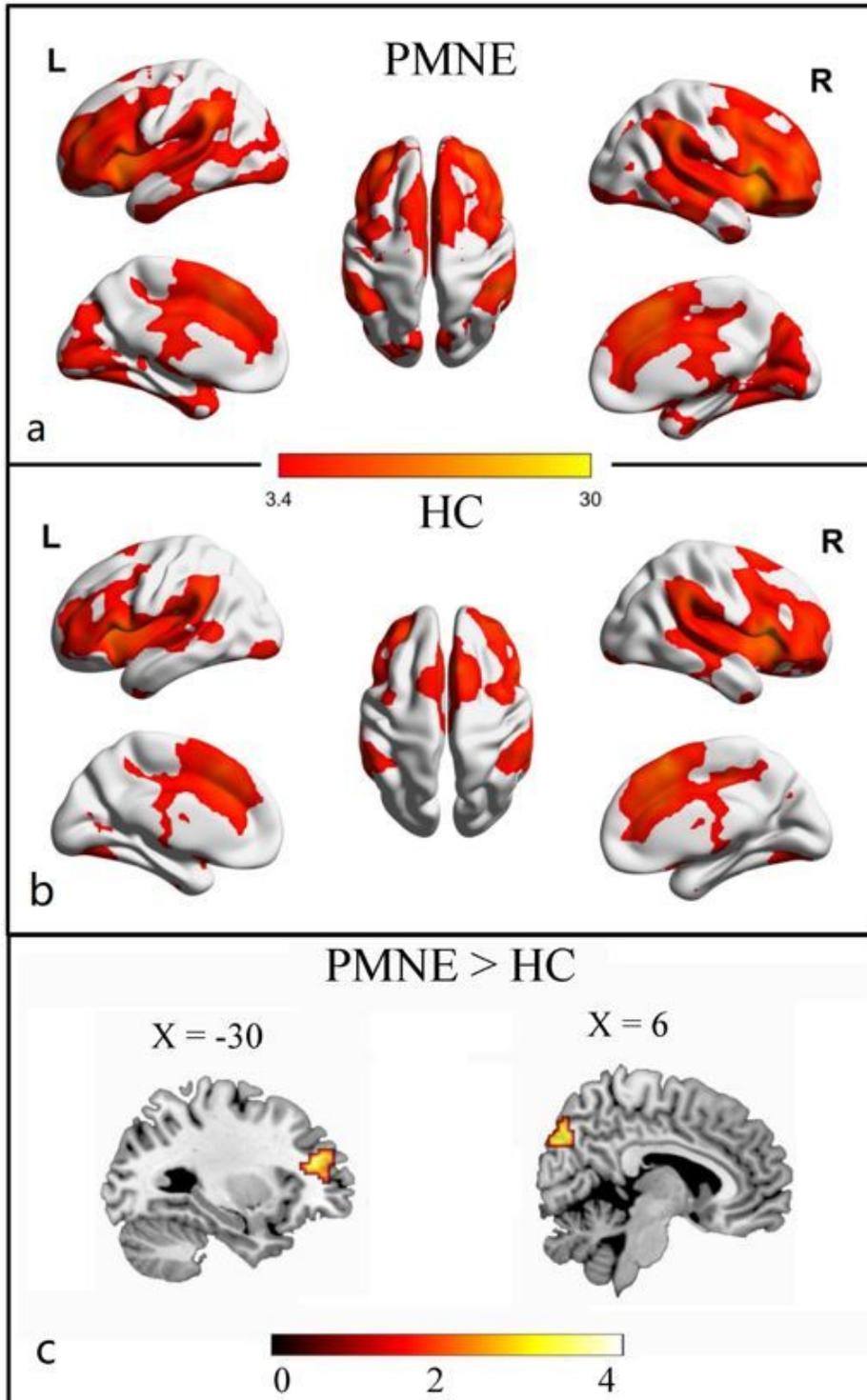


Figure 2

Brain areas showing right IFG related FC network alteration between PMNE patients and HC. FC = functional connectivity; PMNE = primary monosymptomatic nocturnal enuresis; HC = healthy controls; R = right; L = left. Figure a shows the brain areas which were functionally correlated with right IFG in PMNE group; Figure b shows the brain areas which were functionally correlated with right IFG in HC group;

Figure c shows that the right IFG showed increased connectivity with the left middle and inferior frontal gyri and right precuneus extending to the cuneus in the children with PMNE.

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