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Differences in the functional connectivity density of the brain between individuals with growth hormone deficiency and idiopathic short stature

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ABSTRACT

Purpose: The aim of the present study was to investigate the differences in the topological organization of functional brain networks between children with growth hormone deficiency (GHD) and those with idiopathic short stature (ISS).

Methods: Thirty-one children with GHD and fifty-three children with ISS were recruited based on the results of GH stimulation tests. Resting-state fMRI data were acquired from all children. Whole brain functional connectivity density (FCD) analysis and subsequent seed-based functional connectivity analysis were used to explore the differences in functional brain networks between the children with ISS and GHD. Correlation analyses among the results of clinical laboratory examinations, neuropsychological scales and FCD values of different brain regions were applied.

Results: Compared with the ISS group, the GHD group exhibited significantly decreased FCDs in the left postcentral gyrus, right precentral gyrus and left cerebellar lobules 7b and 6. The subsequent functional connectivity analysis found decreased functional connectivity between lobules 7b and 6 of the left cerebellum as well as the left postcentral gyrus and right precentral gyrus in the GHD group compared to that in the ISS group. In addition, the FCD values of region 6 of the left cerebellum in the GHD group were negatively correlated with the scores on the Symptom Checklist-90 and Eysenck Personality Questionnaire. The FCD value of the left postcentral gyrus in children with ISS positively correlated with IGFBP-3 levels and was approximately correlated with IGF-1 levels.

Conclusions: These findings highlight the impact of growth hormone deficiency on the brain network that mainly involves the somatosensory, somatic motor and cerebellum networks, which may contribute to the behavioural problems observed in these children.

1. Introduction

Idiopathic short stature (ISS) and short stature with growth hormone deficiency are the leading causes of short stature in children. The incidence of short stature was approximately 2.5%. Idiopathic short stature accounted for 60%–80% of total short stature, and growth hormone deficiency accounted for 10% of short stature (Cohen et al., 2008; Stochholm and Christiansen, 2011). There is a decrease in growth hormone levels in children with growth hormone deficiency, whereas children with idiopathic short stature exhibit normal growth hormone levels. Growth hormone has recently been shown to affect not only the growing body but also brain maturation (Ashpole et al., 2015; Kreber et al., 2016). Behavioural studies have also shown that children with GHD generally present with psychological and cognitive problems (Deijen et al., 1996; Lasaite et al., 2004; Quitmann et al., 2016). This phenomenon is also found in nonhuman animals (Barcelo et al., 2016). However, the differences in brain function, particularly functional brain networks, between children with GHD and ISS have been poorly

Abbreviations: GHD, growth hormone deficiency; ISS, idiopathic short stature; FCD, functional connectivity density; FC, functional connectivity; EPQ, Eysenck personality questionnaire; CBCL, Achenbach's child behavior checklist; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein 3

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studied. This information is important to improve our understanding of the pathogenesis of the different clinical features between children with GHD and ISS. Our motivation for comparing children with ISS with children with GHD was to explore the role of growth hormone in the dysconnectivity pattern in whole-brain functional networks and to provide evidence for growth hormone replacement therapy.

Growth hormone deficiency has been shown to affect the function of the cerebral cortex. For example, Arwert et al. (2005), 2006 observed stronger local activity, involving supplementary motor and motor cortex, and a slower memory processing speed in children with GHD compared with healthy controls. In addition, a loss of the structural integrity in the corpus callosum and corticospinal tract was related to the cognitive function and behaviours of children with GHD but not children with ISS (Webb et al., 2012). Thus, dysfunctional modulation of brain networks occurs in children with GHD but not children with ISS. However, the differences in functional networks between children with GHD and ISS have rarely been studied.

Recently, resting-state functional connectivity (FC), which measures the temporal correlations of spontaneous fluctuations in brain activity between spatially remote regions, has been extensively used to explore the functional interactions between brain regions. Functional connectivity density (FCD) has several advantages over other traditional parameters, including the lack of a requirement for prior selection of seed regions and the ability to identify functional hubs (densely connected regions) with high sensitivity (Tomasi and Volkow, 2010). Higher FCD values for particular voxels indicate that those voxels are functionally connected to a large number of other brain voxels and suggest that those voxels play more important roles in information processing. In previous neuroimaging investigations, FCD was successfully used to study abnormal functional integration in children with attention attention-deficit/hyperactivity disorder (Tomasi and Volkow, 2012) and anisometropic amblyopia (Wang et al., 2014).

In the present study, we used FCD to investigate the functional connectivity in the brain between children with GHD presenting with a peak GH level < 10 ng/ml and children with ISS presenting with a peak GH level > 10 ng/ml (Anon., 2000). Then, to investigate which connectivities generated the altered FCD values between the two groups, we performed functional connectivity analysis with seed points using abnormal brain regions from the FCD analysis. The correlations between these different brain regions and clinical measures in children with short stature were also measured. We hypothesized that children with GHD would present with abnormal FCDs in the sensorimotor network. Additionally, we determined whether the altered FCD values were correlated with scores on both psychological and behavioural scales.

2. Materials and methods

2.1. Children

Thirty-one children with GHD and fifty-three children with ISS were included in the study. All children with GHD or ISS (aged 4–12 years) were recruited from the Children's Department of Endocrinology at the Second Affiliated Hospital of Wenzhou Medical University. Children were recruited from July 2016 to April 2017. All children were right-handed, as measured by the Edinburgh Inventory (Oldfield, 1971). All children were scanned prior to the growth hormone stimulation test and the growth hormone replacement therapy to avoid the effects of stimulating drugs and growth hormone substitution drugs. As children with short stature present an increased prevalence of behavioural problems compared to controls with normal stature, we chose children with ISS as our 'controls', thereby controlling for the effect of stature and for the effects of GHD on the brain regions associated with emotion. Previous studies have also used children with ISS as a control group for children with GHD (Webb et al., 2012). Children with chronic liver and kidney disease, skeletal system diseases, congenital heart disease, an abnormal thyroid hormone axis, abnormal chromosomes, a history of high fever-induced convulsions, a history of epilepsy, a history of mental illness and abnormal signals in brain T2-weighted images were excluded from the study. The reason we excluded children with a history of mental illness was that the purpose of this study was to study the effects of growth hormone deficiency on the brain, so we had to exclude the effects of mental illnesses on the brain. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University, and parents of the participants provided written informed consent.

2.2. Growth hormone stimulation test and typing of GHD

GH stimulation tests remain the gold standard for diagnosing GH deficiency, but some differences exist between countries (Chesover and Dattani, 2016). The most commonly used GH stimulation test in China is L-dopa followed by clonidine. A GH cut-off value of 10 ng/ml was used to diagnose GHD. A peak GH level > 10 ng/ml indicated no growth hormone deficiency (Anon., 2000), a peak level < 10 ng/ml and > 5 ng/ml indicated partial deficiency, and a peak level < 5 ng/ml indicated complete deficiency. The peak growth hormone level in children with ISS was > 10 ng/ml. ISS was diagnosed in children with a height ≤ 2 standard deviation scores below the average height of children of the same sex, age and race, and these children also presented an annual growth rate of < 5 cm (Cohen et al., 2008).

2.3. Clinical and neuropsychological assessments

Demographic, clinical, and neuropsychological characteristics, including age, sex, IGF-1 levels, IGFBP-3 levels, and scores on the Symptom Checklist-90 and Eysenck Personality Questionnaire (EPQ), were recorded by an experienced neurologist.

2.4. MR image acquisition

All MR imaging examinations were performed with a 3.0-T MR system (3.0 T Discovery MR750, GE Healthcare) by using an 8-channel head coil. T1-weighted high-resolution anatomical images were acquired using a spoiled gradient refocused acquisition sequence: repetition time = 7.68 ms, echo time = 3.43 ms, flip angle = 12 °, field of view = 256 mm^2, slice thickness = 1 mm, layer spacing = 1 mm, and voxel size = 1 mm × 1 mm × 1 mm. Resting-state functional MR imaging data were collected transversely with an echo-planar imaging (EPI) sequence using the following settings: TR = 2000 ms, TE = 30 ms, flip angle = 90°, FOV = 220 mm × 220 mm, slices = 36, in-plane matrix = 64 × 64, thickness = 3 mm, layer spacing = 1 mm, and voxel size = 3.44 mm × 3.44 mm × 4 mm. One hundred eighty volumes were acquired from each subject, resulting in a total scan time of 360 s.

2.5. Data preprocessing

Using SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and Data Processing Assistant for Resting-state fMRI version 4.3 (http://www.restfmri.net), resting-state functional MR imaging data underwent the following preprocessing steps: removal of the first ten volumes, realignment and spatial normalization to the standard MNI (Montreal Neurological Institute) space, spatial smoothing (Gaussian kernel with a full width at a half-maximum value of 6), removal of the linear drift, regression of nuisance signals (mean relative root-mean-square (RMS) deviation for head motion, signals from cerebrospinal fluid and white matter, and the derivatives of each of these signals) and bandpass temporal filtering (0.01-0.08 Hz). The data from all children satisfied the criteria of maximal movements in translation < 3 mm and rotation < 3°. We did not remove the global signal because there is an ongoing debate in the field (Wong et al., 2013)

2.6. Mean motion analysis

Recent studies have demonstrated that head motion may produce both noisy and neuronal effects in fMRI measures (Zeng et al., 2014). Therefore, we calculated the mean relative RMS and mean Frame-wise Displacement power (FD_power) between the two groups from the translation parameters and performed a two-sample *t*-test between the two groups (Jenkinson et al., 2002). The mean motion value represents the mean absolute displacement of each brain volume compared with the previous volume and was estimated from the translation parameters.

2.7. FCD mapping (seed definition)

After the preprocessing procedures, we calculated the functional connectivity density throughout the brains of the GH-deficient group and the ISS group. The functional connectivity density of a voxel represents the number of functional connections of the voxel (Buckner et al., 2009a). The number of functional connections, K(x0), was determined by calculating Pearson's correlation coefficients between time-varying signals at $\times 0$ and those at other voxels using a threshold $R > 0.25$ (Buckner et al., 2009b). We also computed the FCD values using $R > 0.2$ and $R > 0.3$ to ensure that the FCD results were independent from the selection of the R thresholds. The global FCD of a given voxel (x0) was defined as the number of voxels that were functionally connected to voxel x0. The FCD maps of the GHD and ISS groups were rescaled by the individual average FCD (i.e., FCD rescaled = FCD (x, y, z)/mean (FCD)) to reduce the effect of individual variability and increase the normality. The FCD maps were calculated using the DPARSF's scripts.

2.8. Seed-based FC analysis

To examine the validity of the findings of the FCD analysis, we performed a seed-based FC analysis. Based on the FCD maps, regions with abnormal FCD were chosen as seeds. The peak voxel of each seed was selected as a 6-mm radius sphere seed for the seed-based FC analysis using REST software (http://www.restfmri.net). For each child, seed-based FC was calculated as the Pearson correlation coefficients between the seed and other voxels of the whole brain. The correlation coefficients were then Fisher z-transformed to improve the normality of the data, and the seed-based FC maps were generated.

2.9. Statistical analysis

All demographic, clinical, and neuropsychological variables were examined with a two-tailed, two-sample *t*-test. Data on the patients' sex were analysed with a chi-square test.

Voxel-wise comparisons between groups (GHD vs. ISS) were performed using two-sample t-tests on FCD or FC maps. Corrections for multiple comparisons were performed using 3dClustSim in AFNI. The significance level was set to an uncorrected $P < 0.005$, with a voxel number > 30, which corresponded to a corrected $P < 0.05$ (for individual voxels, $P < 0.005$, FWHM = 6 mm, r = 5 mm, iterations = 1000). Analyses were also repeated to include age, sex and the relative mean displacement of head motion as group-level covariates to test the effects of these confounders on the final results. Lastly, we located the abnormal regions by overlapping the statistical t-maps on the AAL and Brodmann templates. Pearson's correlation coefficients were obtained for all correlation analyses.

3. Results

3.1. Group characteristics

Thirty-one children (mean age 9.35 years) with GHD and 53 controls (mean age 9.51 years) with ISS were recruited. All children were right-handed, had no abnormal neurological findings and were in local primary schools. Peak growth hormone was used as a grouping standard between the two groups. No difference in basal growth hormone levels was observed between the two groups ($P = 0.794$). There were no significant differences in IGF-1 ($P = 0.257$) and IGFBP-3 ($P = 0.316$) levels between the GHD group and the ISS group. No significant differences in scores on the Symptom Checklist-90, child behaviour checklist and Eysenck personality questionnaire were found between children with GHD and ISS. The children's characteristics are summarized in Table 1.

Table 1
Demographics and neuropsychological data collected from children with GHD and ISS.

	Growth hormone deficiency	Idiopathic short stature	P-value
Subject characteristics			
Number	31	53	
Mean age (SD, SEM)	9.35 (1.9)	9.51 (1.7)	0.702
Sex; number of females (%)	10(32.3%)	21(39.6%)	0.5
Weight (kg)	25.52 (4.7)	24.38 (4.2)	0.254
Height (m)	1.25 (0.1)	1.24 (0.1)	0.562
Body mass index (kg/m^2)	16.1 (1.1)	15.74 (1.1)	0.114
Basal GH level (μg/l)	0.79 (1.0)	0.84 (1.6)	0.794
Peak growth hormone level (μg/l)	7.49 (2.1)	16.16 (5.3)	0.000
IGF-1 (ng/ml)	114.5 (49.4)	128.5 (56.8)	0.257
IGFBP-3 (ug/ml)	2.69 (1.0)	2.89 (1.0)	0.316
ACTH (pg/ml)	26.24 (27.5)	21.48 (11.8)	0.275
Cortisol (μg/ml)	10.41 (5.6)	10.44 (4.4)	0.979
90 symptom checklist, SLC-90			
Somatization	15.82 (6.7)	16.76 (8.8)	0.606
Obsessive-compulsive symptoms	17.31 (5.3)	17.11 (7.1)	0.896
Interpersonal sensitivity	13.58 (6.4)	13.39 (5.2)	0.885
Depression	18.72 (9.0)	18.63 (8.0)	0.963
Anxiety	15.55 (7.2)	15.26 (7.4)	0.868
Hostility	9.62 (4.3)	9.5 (4.5)	0.909
Phobia	11 (5)	10.72(5)	0.820
Paranoid	8.59 (3.9)	8.96 (4.2)	0.705
Psychoticism	13.97 (7.3)	14.67 (7.2)	0.679
Total score	131.55 (60.8)	134.63 (60)	0.831
Child Behavior Checklist, CBCL			
Activity	4.25 (3.2)	3.34 (2.8)	0.198
Social skills	5.44 (3.9)	5.01 (3.5)	0.631
Learning ability	3.43 (2.5)	3.23 (2.2)	0.714
Total score	19 (18.5)	22.46 (19.1)	0.449
Eysenck Personality Questionnaire, EPQ			
Psychoticism	3.42 (3.2)	2.36 (2.1)	0.101
Extraversion	15.42 (4.9)	15.24 (4.8)	0.879
Neuroticism	7.57 (5.2)	6.64 (4.3)	0.429
Lying	14.31 (4.4)	14.23 (3.5)	0.943

Data are presented as the means (Standard Deviation). Difference in sex distribution used a chi-square test. Other statistical comparisons were performed with a two-tailed two-sample *t*-test.

3.2. Results of the head motion analysis

There was no significant difference between the two groups with regard to mean motion on the basis of a two-sample *t*-test. The t- and p-values for the relative RMS were -0.0031 and 0.9975, respectively. For the mean FD_power, the values were $t = 0.2634$ and $p = 0.7929$.

3.3. Differences in functional connectivity densities between the GH deficiency group and the ISS group

Compared with children with ISS, children with GHD showed significantly decreased FCDs in the left postcentral gyrus, right precentral gyrus and left cerebellar lobules 7b and 6 (Fig. 1 and Table 2). For the FCD analysis using the thresholds of 0.2 and 0.3. The results were consistent with those using a threshold of 0.25 (Figs. 1 and 2).

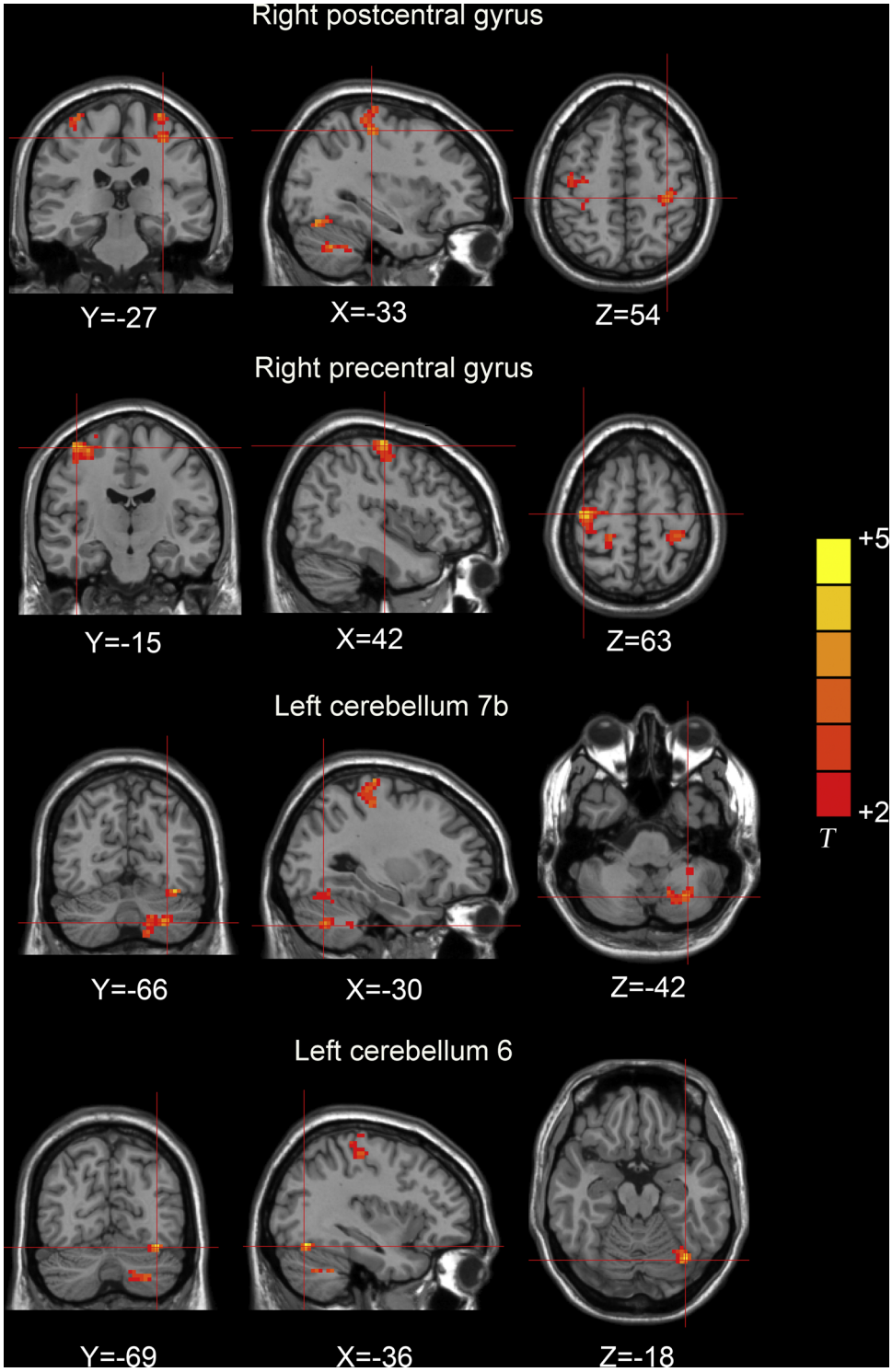

Fig. 1. Brain regions showing significantly decreased FCDs in the GHD group compared with the ISS group. The warm colour indicates that children with GHD have lower global FCDs than the ISS group. The colour bars indicate the t-values of the contrast.

3.4. Associations between FCD values in different brain regions and scores on neuropsychological scales

The FCD values corresponding to the brain regions showing differences between the GHD group and the ISS group were positively correlated with the scores on neuropsychological scales. The FCD values in region 6 of the left cerebellar hemisphere in the GHD group were significantly negatively correlated with four clinical outcomes from the Symptom Checklist-90. They were terror ($r = -0.433$, $p = 0.091$), paranoid ($r = -0.41$, $p = 0.027$), psychotic ($r = -0.387$, $p = 0.038$) and total score ($r = -0.387$, $p = 0.044$). Similarly, FCD values in region 6 of the left cerebellum were significantly negatively correlated with two clinical outcomes of the Eysenck Personality Questionnaire. They were psychoticism ($r = -0.487$, $p = 0.014$) and neuroticism ($r = -0.601$, $p = 0.001$) (Fig. 3).

Table 2
Brain regions with a significantly decreased FCD value in the GH deficiency group compared with the ISS group.

Anatomical regions	Cluster size (voxel)	MNI coordinates x y z	T value
Left postcentral gyrus	90	−33 -27 54	3.9103
right precentral gyrus	206	42 -15 63	4.3350
left cerebellum 7b	90	−30 -66 -42	3.9218
left cerebellum 6	36	−36 -69 -18	4.5664

3.5. Correlations between the IGF-1 axis and FCD values in different brain regions in children with GHD or ISS

The FCD values in the left postcentral gyrus in the ISS group were significantly and positively correlated with IGFBP-3 levels ($r = 0.283$, $p = 0.04$) and approximately positively correlated with IGF-1 levels ($r = 0.262$, $p = 0.058$). The FCD values in region 6 of the left cerebellum in the ISS group were approximately positively correlated with IGF-1 levels ($r = 0.258$, $p = 0.066$) (Fig. 4).

3.6. Alterations in the seed-based functional connectivity analysis in children with GHD or ISS

With the abnormal FCD regions as seed points, the two-sample t-tests revealed significant seed-based FC differences in several related brain regions between the GHD group and the ISS group (Fig. 5).

With the seed point in the left cerebellar lobules 6 and 7b, we found that the GHD group, compared to the ISS group, had decreased FC values, with peak differences in the left postcentral gyrus and right precentral gyrus.

With the seed point in the left postcentral gyrus, we found that the GHD group, compared to the ISS group, had decreased FC values, with peak differences in the left cerebellum crus 1 and left cerebellar lobe 8.

With the seed point in the right precentral gyrus, we found that the GHD group, compared to the ISS group, had decreased FC values, with peak differences in the left cerebellar lobe 6 and right cerebellar lobe 8.

4. Discussion

We aimed to investigate the differences in functional brain networks in children with GHD and ISS. Using whole brain voxel-wise FCD analysis, the GHD group exhibited decreased FCDs in the left postcentral gyrus, right precentral gyrus and Brodmann's area of left cerebellar lobules 7b and 6 compared with the ISS group. In addition, the functional connectivity between the posterior cerebellar lobe and sensorimotor network in the GHD group was significantly weaker than the FC of the ISS group. In addition, the FCD values of region 6 of the left cerebellum in the GHD group were negatively correlated with the scores on the clinical Symptom Checklist-90 and Eysenck Personality Questionnaire. Moreover, the FCD value of the left postcentral gyrus in children with ISS positively correlated with IGFBP-3 levels and was approximately correlated with IGF-1 levels. Taken together, these findings suggest the presence of alterations in brain networks, particularly networks involving the posterior cerebellar lobe and sensorimotor network, in the GHD group compared to the ISS group.

Starting with whole-brain analysis, we initially observed reductions in FCDs in the motor cortex, parietal cortex, and two areas of the posterior lobe of the cerebellum. The former two areas belonged to the sensorimotor network. The latter two regions are closely related to motion and cognition and have been deemed important nodes connected to the cerebral cortex (Buckner et al., 2009a, b). The reduced FCD observed for particular voxels indicated that those voxels were functionally connected to relatively fewer brain voxels and suggested that the function of those voxels in information processing was diminished. More interestingly, the subsequent seed-based connectivity analysis further confirmed our findings and revealed that functional connectivity between lobules 7b and 6 of the left cerebellum and the

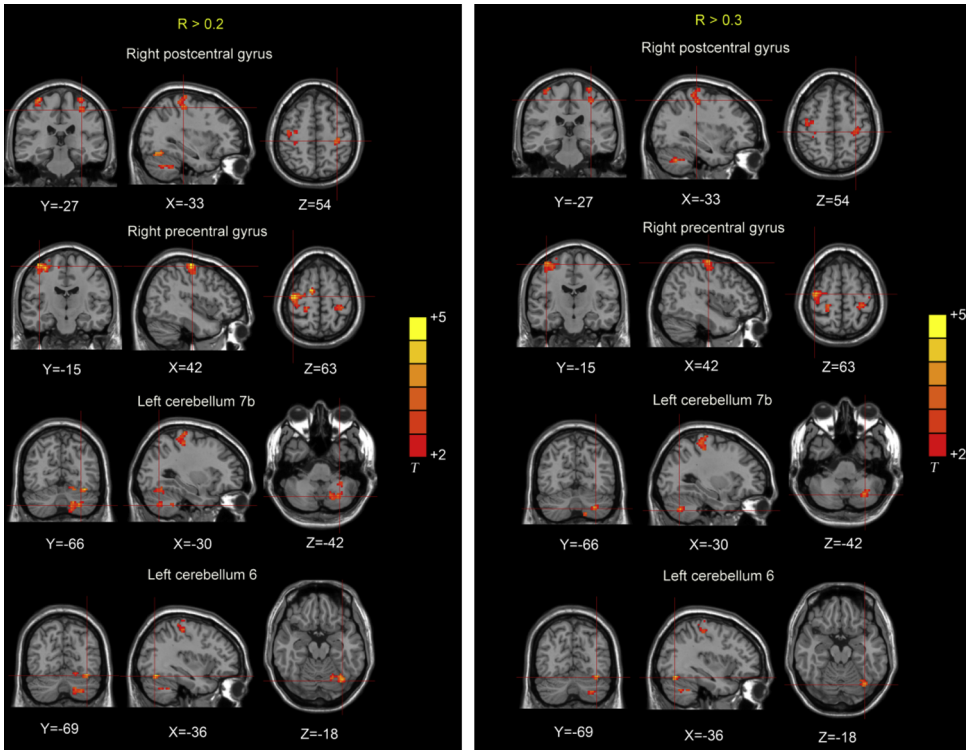

Fig. 2. Brain regions showing significantly decreased FCDs in the GHD group compared with the ISS group using different thresholds. Left image: $R > 0.2$; Right image: $R > 0.3$. The warm colour indicates that children with GHD have lower global FCDs than those in the ISS group. The colour bars indicate the t-values of the contrast.

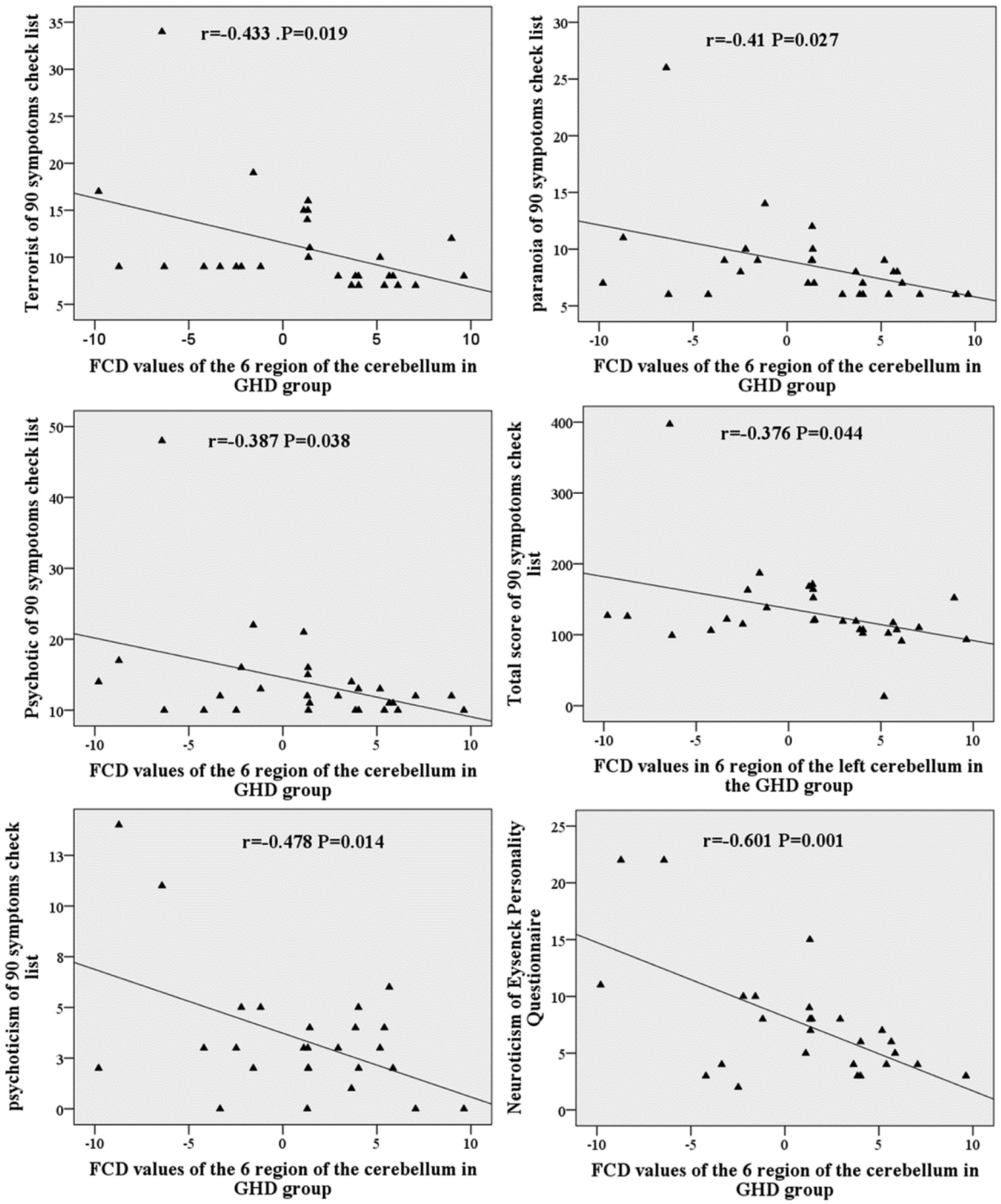

Fig. 3. The relationship between the FCD values in region 6 of the left cerebellar hemisphere and four outcomes (phobia, paranoid, psychoticism, and total score) of the Symptom Checklist-90 and two outcomes (psychoticism and neuroticism) of the Eysenck Personality Questionnaire in children with GHD. Pearson's correlation analysis was used to assess the correlations.

left postcentral gyrus as well as the right precentral gyrus in the GHD group was significantly weaker than in the ISS group. Complex activity in the sensorimotor cortices correlates with activity in cerebellar lobules 6 and 7 (Schlerf et al., 2010; Stoodley, 2012; Stoodley et al., 2012). Based on the anatomical structure, the connection between the cerebrum and cerebellum mainly occurs through the white matter fibre bundle. Growth hormone has been reported to affect dendritic differentiation, synapse formation and the myelination of nerve fibres in brain tissue (Aberg, 2010; Greicius et al., 2009). Therefore, in children with GHD, the integrity of the underlying structural connectivity might be weakened. In fact, previous studies have identified widespread white matter damage in children with GHD based on alterations in fractional anisotropy and mean diffusivity (Webb et al., 2012). Moreover, children with Turner syndrome, which results from the absence of an X chromosome in females and has symptoms of dwarfism and growth hormone deficiency, have been reported to present with alterations in the volume of grey matter in the precentral gyrus, central posterior gyrus and cerebellum (Brown et al., 2002; Marzelli et al., 2011). Taken together, our study provided further evidence to support the deficits of cerebro-cerebellar circuitry in children with GHD.

Furthermore, the FCD values of region 6 of the left cerebellum in GHD were significantly and negatively correlated with multiple

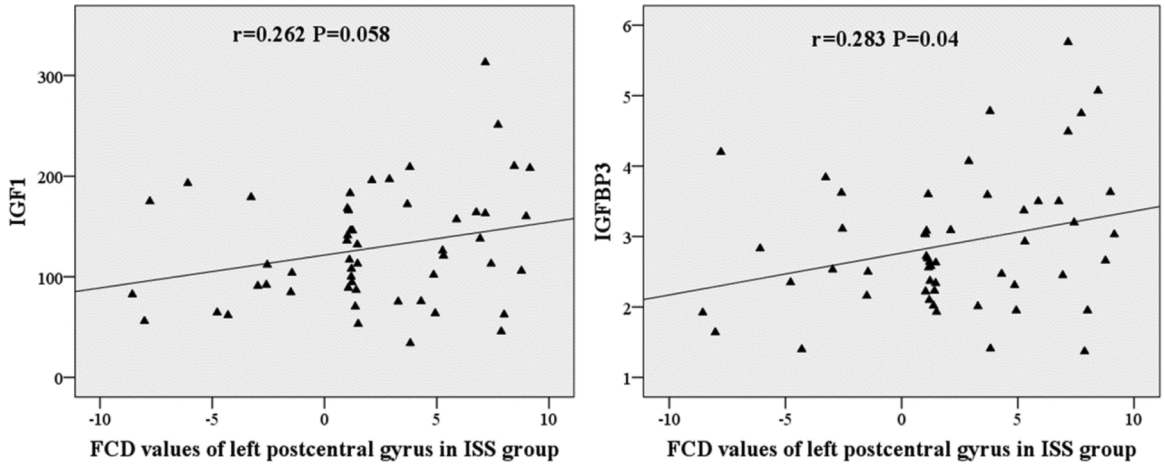

Fig. 4. Correlations between the FCD values in the left postcentral gyrus and IGF-1 and IGFBP-3 levels in children with GHD. Pearson's correlation coefficients were determined. In children with ISS, the FCD values in the left postcentral gyrus were significantly and positively correlated with IGFBP-3 levels (r = 0.243, P = 0.04) and approximately positively correlated with IGF-1 levels (r = 0.242, P = 0.058).

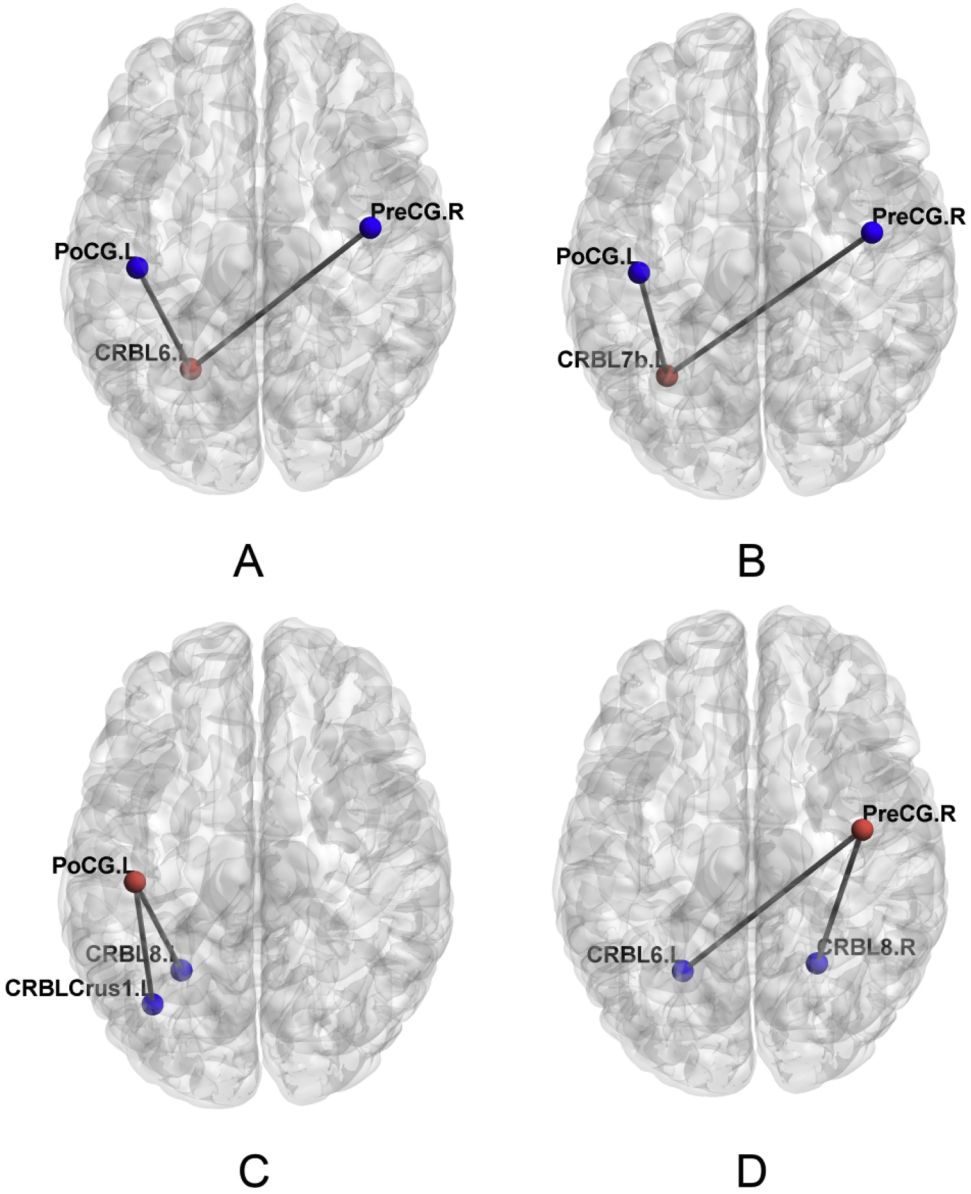

Fig. 5. The red dots represent the seed points, and the pale blue dots represent the remaining brain regions displaying differences in FC. The blue lines represent the functional connections between the seed points and the different brain regions (functional connectivity: GHD < ISS). CRBL6.L: left cerebellum lobule 6. CRBL7b.L: left cerebellar lobule 7b. PoCG.L: left postcentral gyrus. PreCG.R: right precentral gyrus. CRBL8.L: left cerebellar lobule 8. CRBLCrus1.L: left cerebellum crus 1(plotted using Brainnet viewer 1.61 (Xia et al., 2013)) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

outcomes on the Symptom Checklist-90 and Eysenck Personality Questionnaire. The Symptom Checklist-90 is a good unitary measure for assessing psychological status, general distress, and screening for mental disorders, as well as measuring changes in outcome studies. The Eysenck Personality Questionnaire indicates the existence of three major personality traits: neuroticism, extraversion, and psychoticism. There are few articles about the effects of growth hormone deficiency on children's mood. Stabler et al. (1998) reported that children with growth hormone deficiency might have behavioural problems, such as emotional withdrawal, attention deficits, social disorders, and aggression problems. In this study, the Symptom Checklist-90 and the Eysenck Personality Questionnaire were used to analyse the correlation between mental problems and functional connectivity density in children. The results showed that values in area 6 of the left cerebellum were negatively correlated with six results of these scales. This means that the lower the FCD value of the left cerebellar hemisphere area 6, the higher the score on the corresponding scale of particular psychological indicators; the higher the score for a certain index on this psychological scale, the greater the possibility of psychological problems corresponding to this index. We had previously shown that a decrease in the FCD value of a brain area could result in a decrease in the speed of information processing in that area, which might be related to these psychological problems. The cerebellum not only adjusts movement and balance but also plays a role in processing emotions (Adamaszek et al., 2017; Baumann and Mattingley, 2012; Aramburo et al., 2014). In addition, the left cerebellar lobules 7b and 6 are deemed important nodes connected to the cerebral cortex, which we previously mentioned. Animal experiments have also proved that the downregulation of the growth hormone 1 gene in the cerebellum and prefrontal lobe leads to depression-like behavior in mice. Therefore, growth hormone deficiency might be associated with psychological problems related to the decreased FCDs in the cerebellum. In addition, this study suggested that growth hormone deficiency is a risk factor for psychological problems in children, which needs to be a concern of clinicians. Early psychological counselling and growth hormone replacement therapy should be performed in children with growth hormone deficiency. In our study, the FCD value of the left postcentral gyrus in children with ISS positively correlated with IGFBP-3 levels and was approximately correlated with IGF-1 levels. The locations of the brain regions correlated with GH and IGF-1 levels are not continuous, and these regions are often associated with cognition and movement (Adem et al., 1989; Kleinridders, 2016). Based on our findings, the reduced growth hormone levels may affect the function of the postcentral gyrus.

The present findings have the potential to indicate that growth hormone replacement therapy is beneficial for brain development. Our study combined FCD with FC, which is the first application of this approach in the study of brain function in children with short stature. Mutual validation of the two approaches improves the reliability of brain network analysis.

The present study has several limitations. First, this study was cross-sectional in design, and we were not able to identify changes in functional brain networks following the administration of growth hormone replacement therapy in children with GHD. Future longitudinal studies may, therefore, be able to observe changes in brain activities after hormone replacement therapy. Second, fewer people with complete GHD were included in the GH-deficient group. Future studies are needed to balance the number of children with complete GHD and partial GHD. Third, we chose a sphere with a 6-mm radius seed centre in the peak voxel of the abnormal brain regions, which will help other researchers replicate our work. However, seed location may cross anatomical borders and affect the FC results. Finally, only a small number of people in the study were tested with IQ scales; thus, we did not add the IQ scores to the study. In future studies, an IQ scale will be added, and the effects of GHD on cognition and mood will be discussed.

Overall, the present study supports the observed deficits in cerebro-cerebellar circuitry in children with GHD compared to the deficits in children with ISS. These findings highlight the impact of growth hormone deficiency on brain networks that mainly involve the somatosensory, somatic motor and cerebellar networks, which may contribute to the behavioural problems observed in children with GHD.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

Aberg, D., 2010. Role of the growth hormone/insulin-like growth factor 1 axis in neurogenesis. Endocr. Dev. 17, 63–76.
Adamaszek, M., et al., 2017. Consensus paper: cerebellum and emotion. Cerebellum 16 (2), 552–576.
Adem, A., et al., 1989. Insulin-like growth factor 1 (IGF-1) receptors in the human brain: quantitative autoradiographic localization. Brain Res. 503 (2), 299–303.
Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. J. Clin. Endocrinol. Metab. 85 (11), 3990–3993.
Aramburo, C., et al., 2014. Expression and function of growth hormone in the nervous system: a brief review. Gen. Comp. Endocrinol. 203, 35–42.
Arwert, L.I., et al., 2005. Growth hormone deficiency and memory functioning in adults visualized by functional magnetic resonance imaging. Neuroendocrinology 82 (1), 32–40.
Arwert, L.I., et al., 2006. Effects of growth hormone substitution therapy on cognitive functioning in growth hormone deficient patients: a functional MRI study. Neuroendocrinology 83 (1), 12–19.
Ashpole, N.M., et al., 2015. Growth hormone, insulin-like growth factor-1 and the aging brain. Exp. Gerontol. 68, 76–81.
Barcelo, P., et al., 2016. Comparing the behavioural effects of exogenous growth hormone and melatonin in young and old wistar rats. Oxid. Med. Cell. Longev. 2016 5863402.
Baumann, O., Mattingley, J.B., 2012. Functional topography of primary emotion processing in the human cerebellum. Neuroimage 61 (4), 805–811.
Brown, W.E., et al., 2002. Brain development in Turner syndrome: a magnetic resonance imaging study. Psychiatry Res. 116 (3), 187–196.
Buckner, R.L., et al., 2009a. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. J. Neurosci. 29 (6), 1860–1873.
Buckner, R.L., et al., 2009b. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to alzheimer's disease. J. Neurosci. 29 (6), 1860–1873.
Chesover, A.D., Dattani, M.T., 2016. Evaluation of growth hormone stimulation testing in children. Clin. Endocrinol. (Oxf) 84 (5), 708–714.
Cohen, P., et al., 2008. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. J. Clin. Endocrinol. Metab. 93 (11), 4210–4217.
Deijen, J.B., et al., 1996. Cognitive impairments and mood disturbances in growth hormone deficient men. Psychoneuroendocrinology 21 (3), 313–322.
Greicius, M.D., et al., 2009. Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb. Cortex 19 (1), 72–78.
Jenkinson, M., et al., 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17 (2), 825–841.
Kleinridders, A., 2016. Deciphering brain insulin receptor and insulin-like growth factor 1 receptor signalling. J. Neuroendocrinol. 28 (11).
Kreber, L.A., Griesbach, G.S., Ashley, M.J., 2016. Detection of growth hormone deficiency in adults with chronic traumatic brain injury. J. Neurotrauma 33 (17), 1607–1613.
Lasaite, L., et al., 2004. Psychological functioning after growth hormone therapy in adult growth hormone deficient patients: endocrine and body composition correlates. Medicina (Kaunas) 40 (8), 740–744.
Marzelli, M.J., et al., 2011. Neuroanatomical spatial patterns in Turner syndrome. Neuroimage 55 (2), 439–447.
Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9 (1), 97–113.
Quitmann, J.H., et al., 2016. Associations between psychological problems and quality of life in pediatric short stature from patients' and parents' perspectives. PLoS One 11 (4) e0153953.
Schlerf, J.E., et al., 2010. Evidence of a novel somatopic map in the human neocerebellum during complex actions. J. Neurophysiol. 103 (6), 3330–3336.
Stabler, B., et al., 1998. Behavior change after growth hormone treatment of children

with short stature. J. Pediatr. 133 (3), 366–373.
Stochholm, K., Christiansen, J.S., 2011. The epidemiology of growth hormone deficiency. Growth Hormone Related Diseases and Therapy. Springer, pp. 153–167.
Stoodley, C.J., 2012. The cerebellum and cognition: evidence from functional imaging studies. Cerebellum 11 (2), 352–365.
Stoodley, C.J., Valera, E.M., Schmahmann, J.D., 2012. Functional topography of the cerebellum for motor and cognitive tasks: an fMRI study. Neuroimage 59 (2), 1560–1570.
Tomasi, D., Volkow, N.D., 2010. Functional connectivity density mapping. Proc. Natl. Acad. Sci. U. S. A. 107 (21), 9885–9890.
Tomasi, D., Volkow, N.D., 2012. Abnormal functional connectivity in children with attention-deficit/hyperactivity disorder. Biol. Psychiatry 71 (5), 443–450.
Wang, T., et al., 2014. Abnormal functional connectivity density in children with anisometropic amblyopia at resting-state. Brain Res. 1563 (4), 41–51.
Webb, E.A., et al., 2012. Effect of growth hormone deficiency on brain structure, motor function and cognition. Brain 135 (Pt 1), 216–227.
Wong, C.W., et al., 2013. The amplitude of the resting-state fMRI global signal is related to EEG vigilance measures. Neuroimage 83, 983–990.
Xia, M., Wang, J., He, Y., 2013. BrainNet Viewer: a network visualization tool for human brain connectomics. PLoS One 8 (7) e68910.
Zeng, L.-L., et al., 2014. Neurobiological basis of head motion in brain imaging. Proc. Natl. Acad. Sci 201317424.