Supplementary

Methods

Behavioral correlation analyses

To assess whether symptom severity was correlated with regional activities overall and in each cohort in the autism group, we calculated the Spearman rank-order correlation or partial correlation in each age cohort with z-values and three questionnaire scores as variables in each of our significant clusters. The questionnaires used were the Autism Behavior Checklist (ABC) (38), Aberrant Behavior Checklist (39) and Autism Spectrum Screening Questionnaire (ASSQ) (40,41), which are all parent-report questionnaires. The ABC is a checklist to identify individuals with a high level of autistic behaviors, which contains 57 items grouped into 5 subscales (sensory, social, body/object use, language, and self-help). The Aberrant Behavior Checklist is an informant rating instrument that contains 58 items on 5 subscales (irritability, lethargy/social withdrawal, stereotypic behavior, hyperactivity/noncompliance, and inappropriate speech) using a "0" (not true) to "3" (almost always true) point Likert scale. The ASSQ is a 27-item autism screening tool for 7-16 years old individuals that contains 3 subscales (social interaction, communication problem, and restricted and repetitive behavior) using a "0" (not true) to "2" (almost always true) Likert scale. All three questionnaires were identified to have good reliability and validity in Chinese ASD individuals (42-44). Given the cross-sectional nature of the present study and potential sampling bias, the relationship between age and the scores of the ABC, Aberrant Behavior Checklist and ASSQ were examined separately using partial correlation analyses. For the data that do not fit a Gaussian distribution, Spearman rank-order correlation was employed. No significant age effect was shown in any scale assessment within the autism group ($P>0.05$).

Results

For the whole autism group, there was no significant correlation between symptom severity and ALFF z-values in the two clusters (all $P>0.05$). In the autism adolescence cohort, ALFF z-values in the Caudate cluster and ABC total score were significantly related ($r=0.492$, $P=0.045$), but this correlation did not survive Bonferroni correction for multiple comparisons ($P=0.003$).

Discussion

Whether or to what extent striatal regional activity abnormalities are related to autistic symptoms in autism has not been answered in previous studies. To answer this question, we investigated the relationship between atypical ALFF values in Caudate and Putamen clusters with symptom severity evaluated by the ABC, Aberrant Behavior Checklist and ASSQ in the whole group and in each age stratification group of autistic individuals. Our results suggested that spontaneous activities near the dorsal striatum were positively correlated with the total ABC scores of autism adolescents, but this result did not survive multiple comparisons correction. The dorsal striatum, which is a crucial subcortical region in the subcortico-cortical circuit, is associated with cognitive, sensorimotor and reward functions, which are all aberrant in ASD individuals, particularly repetitive or stereotyped behaviors and social reward processing (26,33). Task-related R-fMRI studies have reported that the severity of those symptoms increased with decreased activation in the dorsal striatum, especially in the Caudate and Putamen (45). Our study did not replicate the significant correlation between abnormal ALFF values and restricted and repetitive behaviors as reported in previous studies; however, we observed a marginally significant correlation between ALFF z-values in th caudate cluster and the ABC total score in the adolescent autism cohort (uncorrected) (34,46). To determine why our study obtained results that were inconsistent with prior studies, insufficient sample size should be considered. Therefore, it is necessary to enlarge the sample size to explore the relationship between ASD symptom severity and DALFF values in the future.

However, it is worth noting that there were contradictory results in ASD symptom correlation analyses in prior studies. For example, Charman and his colleagues showed higher ASD symptom severity in adults than in adolescents in a large multicenter, multidisciplinary observational study (47). However, Langen and colleagues observed that repetitive behaviors were negatively correlated with caudate nucleus volume, and caudate nucleus volume was negatively related to age in ASD patients (26). So far, there is no explanation for those inconsistent findings. Hence, we assume that higher regional spontaneous activity and/or overconnectivity in children with ASD were probably only related to compensatory mechanisms

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but had no direct relation with clinical symptoms. In addition, we assume that regional activity might affect the outcome; that is, other unpredictable factors might work together to influence the symptoms. Consequently, it is necessary to explore the correlations between regional activity, functional connectivity, and autistic traits in further studies.

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Table S1 Diagnostic main effect of significant clusters using ALFF with GSR

	Cluster Size (Voxels)	Peak MNI				Max intensity (z)	Region(s)[a,c]
		x	y	z	Region[b]		
Cluster 1	48	21	12	-9	Putamen_R	-4.763	Right Cerebrum, Right Putamen, Lentiform Nucleus, Right Caudate, Frontal Lobe
Cluster 2	60	27	-12	-9	-	4.845	Right Cerebrum, Right Putamen, Lentiform Nucleus, Right Pallidum

ALFF: amplitude of low-frequency fluctuations. GSR: Global signal regression. [a]Cluster size ≥5 voxels; [b]Brain regions as defined with Anatomical Automatic Labeling (AAL); [c]Brain regions as defined with Harvard-Oxford Subcortical Atlas and/or Harvard-Oxford Cortical Structural Atlas.

Table S2 Diagnostic group-by-age interactions effect of significant clusters using ALFF with GSR

	Cluster Size (Voxels)	Peak MNI				Max intensity (z)	Region(s)[a,c]
		x	y	z	Region[b]		
Cluster 1	252	27	6	-3	Putamen_R	-4.813	Right Cerebrum, Right Putamen, Lentiform Nucleus, Right Pallidum, Frontal Lobe, Claustrum, Right Caudate, Right Insula.

ALFF: amplitude of low-frequency fluctuations. GSR: Global signal regression. [a]Cluster size ≥5 voxels; [b]Brain regions as defined with Anatomical Automatic Labeling (AAL); [c]Brain regions as defined with Harvard-Oxford Subcortical Atlas and/or Harvard-Oxford Cortical Structural Atlas.

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Table S3 Demographic information in each age cohort.

	Autism	TD	$t/\chi^2/z$	P value
Childhood (6.0-11.9y)	n=27	n=22		
Gender (male: female)	23:4	14:8	3.04	0.081
Age [a]	8.7±1.7	9.0±1.6	-0.637	0.529
Full Scale IQ [a]	98±16	109±10	-2.658	0.011
Verbal IQ [a]	103±19	111±14	-1.616	0.113
Performance IQ [a]	92±15	104±11	-2.973	0.005
Head motion (mean FD) [b]	0.147	0.096	-1.286	0.198 [c]
Adolescence (12.0-17.9y)	n=27	n=18		
Gender (male: female)	23:4	12:6		0.166 [d]
Age [a]	14.5±1.5	14.5±1.6	-0.001	0.999
Full Scale IQ [a]	103±16	117±11	-3.144	0.003
Verbal IQ [a]	109±18	119±14	-1.871	0.068
Performance IQ [a]	94±15	111±11	-4.028	<0.001
Head motion (mean FD) [b]	0.116	0.059	-2.062	0.039 [c]
Adulthood (18.0-30.0y)	n=11	n=15		
Gender (male: female)	10:1	14:1		1.000 [d]
Age [a]	21.6±2.9	22.5±3.6	-0.695	0.494
Full Scale IQ [a]	115±17	123±6	-1.697	0.103
Verbal IQ [a]	116±18	120±7	-0.816	0.423
Performance IQ [a]	110±13	120±9	-2.350	0.027
Head motion (mean FD) [b]	0.108	0.048	-2.621	0.008 [c]

IQ, intelligence quotient; mean FD, mean frame-wise displacement; TD, typical development; SD, standard deviation. ALFF: amplitude of low-frequency fluctuations. GSR: Global signal regression. [a]Mean ± SD; [b]Median; [c]Asymptotic significance was displayed using nonparametric test; [d]Fisher's Exact Test.

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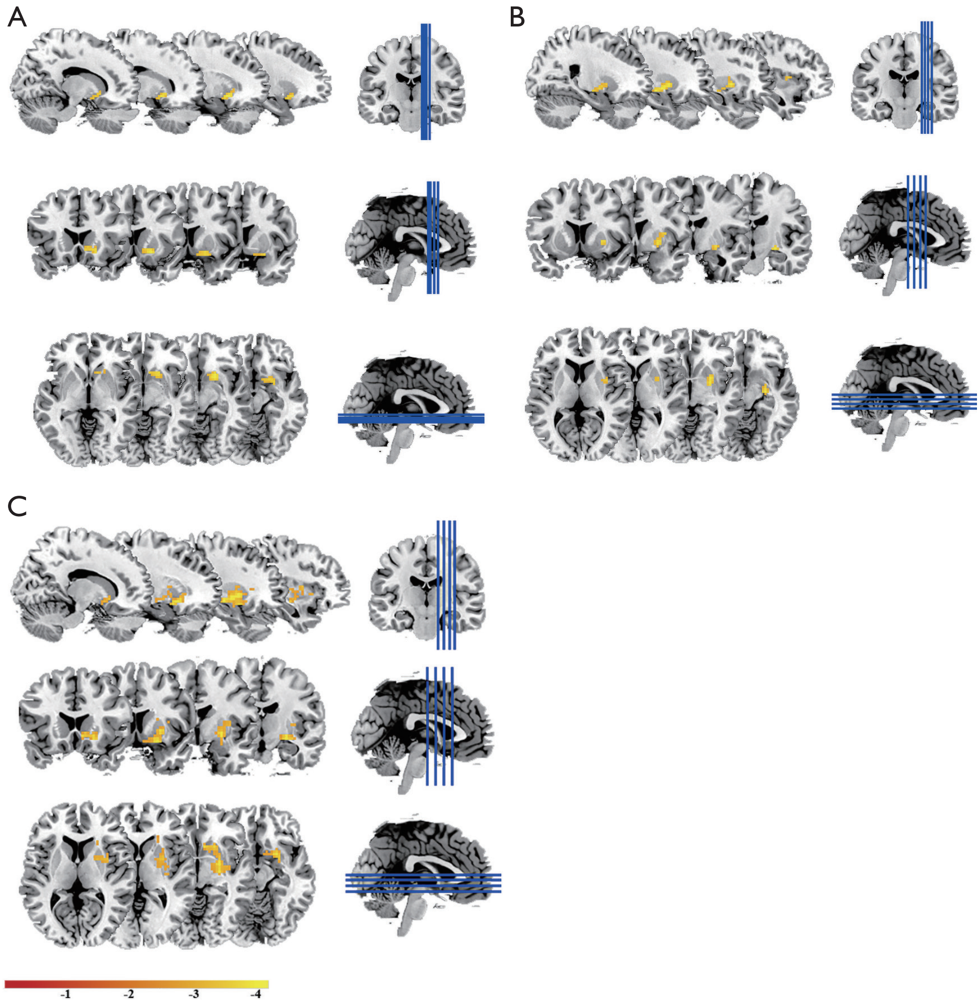

Figure S1 Figure A and B show two clusters of diagnostic group main effect (yellow) on regional spontaneous brain activity with GSR respectively (permutation test with TFCE, P<0.05, corrected). C shows diagnostic group-by-age interactions effects on regional spontaneous brain activity with GSR (permutation test with TFCE, P<0.05, corrected). Slices were generated using MRIcroN software (sagittal, coronal and axial plane). TFCE: threshold-free cluster enhancement.

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