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Trends in regional morphological changes in the brain after the resolution of hypercortisolism in Cushing's disease: a complex phenomenon, not mere partial reversibility

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Abstract

The adverse effects of hypercortisolism on the human brain have been highlighted in previous studies of Cushing's disease (CD). However, the relative alterations in regional hypercortisolism in the brain remain unclear. Thus, we investigated regional volumetric alterations in CD patients. We also analyzed the associations between these volumetric changes and clinical characteristics. The study participants comprised of active CD ($n = 60$), short-term-remitted CD ($n = 28$), and long-term-remitted CD ($n = 32$) patients as well as healthy control subjects ($n = 66$). Gray matter volumes (GMVs) were measured *via* voxel-based morphometry. The GMVs of substructures were defined using the automated anatomical labeling (AAL) atlas. Trends toward normalization in GMV were found in most brain substructures of CD patients. Different trends, including enlarged, irreversible, and unaffected, were observed in the other subregions, such as the amygdala, thalamus, and caudate. Morphological changes in GMVs after the resolution of hypercortisolism are a complex phenomenon; the characteristics of these changes significantly differ within the brain substructures.

Key Words
- Cushing's disease
- gray matter volumes
- regional alteration
- hypercortisolism

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Introduction

Cushing's disease (CD) is a rare disorder of chronic hypercortisolism exposure caused by an adrenocorticotrophic hormone (ACTH)-secreting pituitary adenoma (1). Hypercortisolism can induce a variety of physical manifestations and psychiatric symptoms in CD. Tumor resection can not only correct hypercortisolism but can also achieve partial recovery of these symptoms (2). Thus, CD could represent a unique human model for examining hypercortisolism-related alterations, reversibility of disease-related changes after the resolution of the condition, and the relationships between these alterations and clinical characteristics (3).

The detrimental effects of hypercortisolism on the brain were first highlighted in early autopsy and pneumoencephalography reports (4, 5). The introduction of MRI enabled a more accurate assessment of brain structures. Previous MRI studies have already demonstrated the relationship between hypercortisolism and whole-brain damage (2, 6). However, the associations between regional structural alterations and hypercortisolism remain

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controversial. For instance, Starkman *et al.* demonstrated that the hippocampal volumes of CD patients were smaller than those of normal subjects reported in the literature (7). Their longitudinal study also demonstrated significant recovery of hippocampal volumes 1 year after curative surgery (8). However, another longitudinal study found only an increase in caudate volume 12 months after the resolution of the condition (9). In addition, although volumes of the left amygdala of active CS patients negatively correlated with depression scores, differences could only be found in right (but not left) amygdala volumes when compared with controls (10).

Our total brain volume analysis demonstrated trends of a partially reversible reduction in total gray matter volume (GMV) in CD patients (11, 12). However, previous studies found only partially reversible GMVs reduction in particular subregions, such as the hippocampus (8). Furthermore, segmented GMVs of previous voxel-based morphometry (VBM) studies demonstrated inconsistent results in other subregions, such as the amygdala, frontal lobe, and caudate (2). In addition, several fMRI studies have demonstrated alterations in the frontal lobe and thalamus (13, 14, 15). Nevertheless, partial reversibility of the changes in each brain subregion after the resolution of hypercortisolism remains controversial.

Moreover, long after a biochemical cure, CD patients still have elevated cardiovascular morbidity and mortality, a higher prevalence of psychopathology, and impaired cognitive function (16). Moreover, irreversible brain alterations have long been demonstrated in previous studies of long-term-remitted CD patients (12, 17). Previous studies have tended to explore long-term reversibility after the resolution of hypercortisolism. However, a recent longitudinal study demonstrated significant reversibility of GMVs in short-term-remitted CD patients (3 months after the resolution of hypercortisolism) (17). Our research group also demonstrated trends of potential reversibility in GMV changes in short-term-remitted CD patients (mean remission time of almost 2 months) (11). To our knowledge, there is little information on the similarities and differences among active, short-term-remitted, and long-term-remitted CD patients that have been evaluated by modern, sophisticated analytical tools.

In the present study, based on the VBM technique, we retrospectively evaluated GMVs in active CD, short-term-remitted CD, and long-term-remitted CD patients and healthy controls (HCs) in greater detail using the automated anatomical labeling (AAL) atlas. We conducted an explorative whole-brain analysis to detect possible structural changes in areas outside these *a priori*-defined regions of interest (ROI). In addition, we aimed to explore the associations between structural changes and clinical characteristics. Detailed knowledge of the nature of morphological changes of gray matter could greatly help in determining the underlying mechanism of hypercortisolism in the human brain.

Materials and methods

Participants

CD patients were recruited during their routine visits to Rui-Jin Hospital. HCs were recruited *via* social media and online advertisements and matched to the CD patients based on sex, age, and education. All individuals provided written informed consent before the study participation. The participants were excluded if they had a history of drug and/or alcohol abuse, neurological problems, a history of brain lesion, and contraindications to MRI and are left-handed. A total of 186 study subjects were included, all right-handed with at least 9 years of education. According to the remission duration of CD, we included three groups of CD patients and one group of HCs. These groups comprised of 60 patients with active CD (aCD), 28 patients with short-term-remitted CD (S-rCD) (remission time, 17–135 days; mean ± SD., 62.14 ± 35.09 days), 32 patients with long-term-remitted CD (L-rCD) (remission time, 198–1472 days; mean ± SD., 709.75 ± 385.47 days), and 66 HCs. This study was approved by the Institutional Review Board of Rui-Jin Hospital. CD and its etiology were confirmed by an elevated 24-h urinary free cortisol (UFC) level, bilateral petrosal sinus sampling, absence of a blunted circadian rhythm of cortisol secretion, and other clinical features (18). CD remission was confirmed in all remitted CD patients by normal UFC after surgery.

Clinical characteristics

Clinical characteristics, including 24-h UFC levels, serum cortisol and ACTH levels, BMI, weight, blood pressure, and plasma glucose level, were evaluated in all CD patients. Disease duration was calculated from the earliest point at which clinical signs were noted in the patient's history to the time of cortisol normalization. Remission duration was calculated from the date of surgery. The medical histories of all study subjects were recorded using a standardized questionnaire.

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MRI acquisition and processing

The subjects were scanned using a 3.0T Philips Ingenia MRI scanner and a 3.0T GE Signa Excite HD (Chicago, IL, USA) MRI scanner. High-resolution T1-weighted images were obtained using three-dimensional brain volume imaging sequences with the following settings: repetition time, 5.552 ms; echo time, 1.752 ms; field of view, 256 × 256 mm; flip angle, 12°; 1 sagittal acquisition; matrix, 256 × 256; NEX, 1; slices, 96 thickness, 1.0 mm with no interslice gap; and bandwidth, 244.141 Hz.

The Computational Anatomy Toolbox (CAT12; http://dbm.neuro.uni-jena.de/cat12/) in MATLAB (R2012b; MathWorks, Natick, MA, USA) was used to analyze T1-weighted images. We conducted brain extraction and spatial normalization and obtained GMVs after brain segmentation according to the standard template. In addition, we corrected the influence of spatial normalization. We defined the total GMV as the summation of the GMVs of all voxels and analyzed GMVs in the ROIs (i.e. the hippocampus, amygdala, cerebellum, frontal lobe, thalamus, and caudate). To provide a complete view of alterations in the whole brain, we also used the AAL atlas to parcellate the GMV maps into all other brain subregions (ROIs) (19).

Statistical analysis

We analyzed the demographic and clinical characteristics of the four groups. Differences in age and education were determined *via* one-way ANOVA. Sex differences were determined using the chi-squared test. Among the three groups of CD patients, we employed one-way ANOVA to compare the patients' BMI, weight, plasma glucose level, systolic pressure, diastolic pressure, serum cortisol levels, 24-h UFC level, ACTH level, and disease duration.

To examine gray matter changes during hypercortisolism exposure and after the resolution of the condition, the four groups' total GMVs, as well as 116 AAL-based GMVs, were compared *via* one-way ANOVA. Age, sex, education level, differences in MRI, and total intracranial volume (TIV) were used as covariates. Furthermore, we calculated the partial correlation between each clinical characteristic and each GMV measurement while controlling the abovementioned covariates. All data analyses were conducted using the SPSS software (version 22.0; SPSS). For all statistical tests, a two-sided *P*-value of 0.05 was used, unless Bonferroni correction for multiple comparisons was indicated. Moreover, we calculated the partial correlation between each GMV measure and each clinical characteristic, including 24-h UFC levels, serum cortisol level, ACTH level, BMI, weight, blood pressure, plasma glucose level, and disease duration.

To investigate the potential reversibility of GMV changes in CD patients, one-way ANOVA of regional GMVs from longitudinal MRI data (including active and short-term remission stages for each CD patient) and data of age-, sex-, and education-matched HC subjects were calculated. A total of 11 patients and 11 HC subjects were included in the preliminary longitudinal study. Partial correlations between these GMVs and clinical characteristics were also calculated.

Results

Demographic and clinical characteristics

The demographic data, clinical characteristics, hormone levels, GMVs, and TIVs of all four groups are presented in Table 1.

No significant differences were observed among the four groups in terms of age, sex, or education level. Moreover, there were no significant differences among the three CD groups in terms of disease duration.

All adenomas of CD patients were smaller than 10 mm. All CD patients were treated with transsphenoidal surgery without radiotherapy or bilateral adrenalectomy. About 8 of 60 active CD patients and 1 short-term-remitted CD patient had previously undergone surgery. One long-term-remitted CD patient used steroidogenesis inhibitors to achieve eucortisolism after surgery. CD remission was confirmed by normal UFC and adrenal insufficiency in all remitted CD patients following surgery or medical treatment.

Compared with the two remitted CD groups, significantly higher BMI, body weight, plasma glucose level, blood pressure, 24-h UFC level, serum cortisol level, and adrenocorticotropin level were observed in active CD patients. All short-term-remitted CD patients and 21 of all 32 long-term-remitted CD patients were substituted with hydrocortisone.

ANOVA and correlation analysis of total GMVs

The total GMV analysis of this study was similar to that of our previous total brain volume study, which included some of the participants of the current study (12). A significant difference ($P < 0.001$) was observed in the current study's ANOVA of total GMV. HCs had the largest total GMV

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Table 1 Demographics and clinical characteristics of study patients and healthy control subjects.

Characteristics	aCD (n = 60)	S-rCD (n = 28)	L-rCD (n = 32)	HC (n = 66)	P value
Current age (years)	42.28 ± 11.49	38.89 ± 14.59	44.50 ± 11.43	45.32 ± 14.59	0.153[a]
Gender (male/female)	10/50	5/23	3/29	18/48	0.181[b]
Education (years)	12.67 ± 3.07	12.29 ± 2.75	11.34 ± 3.62	13.02 ± 3.02	0.092[a]
BMI	26.09 ± 4.85	26.23 ± 4.13	23.73 ± 3.54	–	0.032[a, d]
Weight (kg)	68.84 ± 14.33	69.49 ± 11.18	60.73 ± 9.88	–	0.007[a, e]
Plasma glucose (mmol/L)	6.56 ± 2.29	5.46 ± 1.18	5.12 ± 1.23	–	0.001[a, e]
Systolic pressure (mmHg)	143.88 ± 19.57	129.39 ± 21.14	122.25 ± 20.36	–	<0.001[a, f]
Diastolic pressure (mmHg)	90.78 ± 13.40	86.86 ± 13.17	81.59 ± 13.90	–	0.009[a,e]
Estimated illness duration (months)	78.78 ± 108.86	55.86 ± 68.05	59.44 ± 56.23	–	0.429[a]
Remission time (days)	–	62.14 ± 35.09	709.75 ± 385.47	–	<0.001[c, f]
Hydrocortisone replacement (mg)	–	39.21 ± 18.49	16.41 ± 16.63	–	<0.001[c, f]
Urinary free cortisol (μg/24 h)	781.49 ± 869.91	120.82 ± 125.28	103.93 ± 142.13	–	<0.001[a, f]
Plasma cortisol (08:00 h) (μg/dL)	26.54 ± 15.24	2.46 ± 4.10	4.29 ± 7.22	–	<0.001[a, f]
Plasma cortisol (16:00 h) (μg/dL)	21.85 ± 10.54	4.87 ± 4.15	5.31 ± 5.32	–	<0.001[a, f]
Plasma cortisol (00:00 h) (μg/dL)	21.56 ± 14.54	3.82 ± 3.80	4.20 ± 5.66	–	<0.001[a, f]
Plasma ACTH (pg/mL)	98.45 ± 69.03	14.31 ± 14.59	15.78 ± 16.89	–	<0.001[a, f]
Gray matter volumes (GMVs) (cm^3)	564.79 ± 30.01	585.69 ± 30.09	602.46 ± 31.23	614.14 ± 31.12	<0.001[a, f]
Total intracranial volumes (cm^3)	1387.68 ± 95.32	1421.99 ± 97.09	1409.68 ± 100.78	1446.38 ± 98.91	0.011[a, d]
BMI	26.09 ± 4.85	26.23 ± 4.13	23.73 ± 3.54	–	0.032[a, d]
Weight (kg)	68.84 ± 14.33	69.49 ± 11.18	60.73 ± 9.88	–	0.007[a, e]
Serum glucose (mmol/L)	6.56 ± 2.29	5.46 ± 1.18	5.12 ± 1.23	–	0.001[a, e]
Systolic pressure (mmHg)	143.88 ± 19.57	129.39 ± 21.14	122.25 ± 20.36	–	<0.001[a, f]
Diastolic pressure (mmHg)	90.78 ± 13.40	86.86 ± 13.17	81.59 ± 13.90	–	0.009[a, e]
Estimated illness duration (months)	78.78 ± 108.86	55.86 ± 68.05	59.44 ± 56.23	–	0.429[a]
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Plasma cortisol (00:00 h) (μg/dL)	21.56 ± 14.54	3.82 ± 3.80	4.20 ± 5.66	–	<0.001[a, f]
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Systolic pressure (mmHg)	143.88 ± 19.57	129.39 ± 21.14	122.25 ± 20.36	–	<0.001[a, f]
Diastolic pressure (mmHg)	90.78 ± 13.40	86.86 ± 13.17	81.59 ± 13.90	–	0.009[a, e]

Values are expressed as mean ± s.d.

[a]The P value for current age, education, BMI, weight, serum glucose level, systolic pressure, diastolic pressure, disease duration, and hormone level among groups was obtained by one-way ANOVA; [b]The P value for gender distribution in the four groups was obtained by chi-square test; [c]The P value for remission time and dose of hydrocortisone replacement between the two rCD groups was obtained by two sample t-test; [d]$P < 0.05$; [e]$P < 0.01$; [f]$P < 0.001$. aCD, patient group with active CD; HC, healthy control group; L-rCD, patient group with long-term remitted CD; S-rCD, patient group with short-term remitted CD.

(614.14 ± 31.12 mm^3) among the four groups, whereas active CD patients had the smallest total GM volume (564.79 ± 30.01 mm^3) among the four groups. The total GM volumes of the two remitted CD groups (585.69 ± 30.09 mm^3 and 602.46 ± 31.23 mm^3) were between those of the HC group and the active CD group. Furthermore, significant and positive correlations were found between the total GMVs and remission time in the short-term-remitted CD group (R=0.591, P=0.003). These results indicated that trends of reversible changes in the total GMV occurred shortly after the resolution of hypercortisolism (Fig. 1 and Table 1).

The trends of GMV changes in ROIs

ANOVA and *post hoc* analysis of GM volumes among four groups were performed. The correlation analysis between remission time and GM volumes was also performed in two remitted CD groups. Four patterns could be found in brain subregions under chronic exposure to hypercortisolism. The details of the differences in the GMVs of ROIs among the four groups are presented in Table 2 and in Supplementary Tables 1, 2 and 3 (see section on supplementary materials given at the end of this article).

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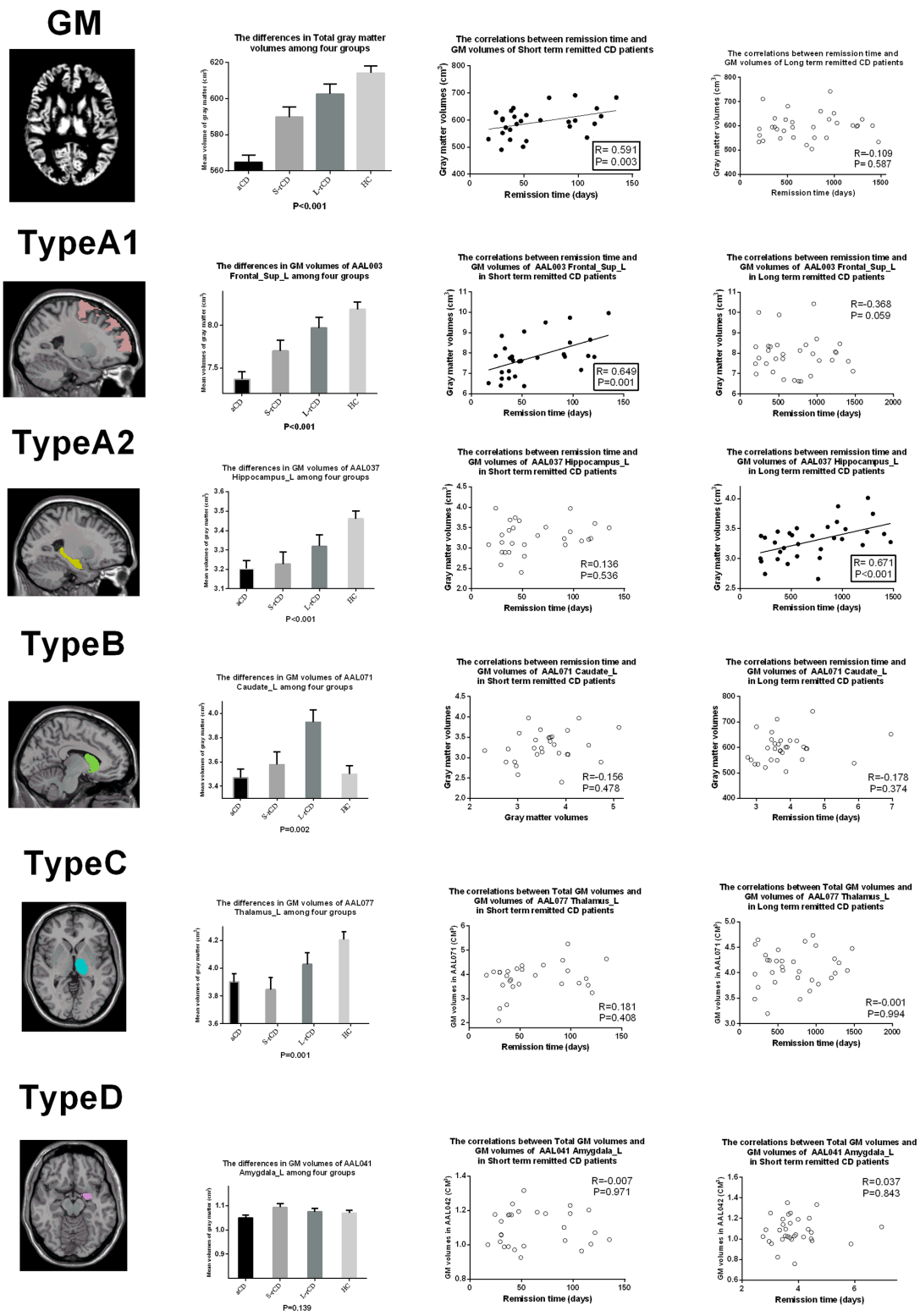

Figure 1

Group differences in gray matter volume and correlations with remission time. 1-GM Group differences and correlation analysis in total GM volume; 1-type A1 group differences and correlation analysis in type A1; 1-type A2 group differences and correlation analysis in type A2; 1-type B group differences and correlation analysis in type B; 1-type C group differences and correlation analysis in type C; 1-type D group differences and correlation analysis in type D. CD, Cushing's disease; GM, gray matter; aCD, patients with active CD; S-rCD, patients with short-term-remitted CD; L-rCD, patients with long-term-remitted CD; HC, healthy controls.

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Table 2 GM volumes of ROIs among study patients and healthy control subjects (samples).

AAL template	Regions of interest	aCD ($n = 60$)	S-rCD ($n = 28$)	L-rCD ($n = 32$)	HC ($n = 66$)	*P* values
Type A: trend toward normalization (number of ROIs = 87)						
003_Frontal_Sup_L	Left superior frontal gyrus	7.368 ± 0.674	7.701 ± 0.677	7.971 ± 0.701	8.189 ± 0.699	<0.001[a]
Post hoc analysis	aCD << S-rCD	aCD <<< L-rCD	aCD <<< HC	S-rCD = L-rCD	S-rCD << HC	L-rCD < HC
	$P = 0.008$[b]	$P < 0.001$[a]	$P < 0.001$[a]	$P = 0.334$	$P = 0.001$[b]	$P = 0.039$[c]
004_Frontal_Sup_R	Right superior frontal gyrus	8.244 ± 0.736	8.628 ± 0.741	9.037 ± 0.764	9.155 ± 0.745	<0.001[a]
Post hoc analysis	aCD << S-rCD	aCD <<< L-rCD	aCD <<< HC	S-rCD = L-rCD	S-rCD << HC	L-rCD = HC
	$P = 0.006$[b]	$P < 0.001$[a]	$P < 0.001$[a]	$P = 0.182$	$P = 0.002$[b]	$P = 0.120$
037_Hippocampus_L	Left hippocampus	3.204 ± 0.325	3.227 ± 0.328	3.319 ± 0.339	3.460 ± 0.341	<0.001[a]
Post hoc analysis	aCD = S-rCD	aCD = L-rCD	aCD <<< HC	S-rCD = L-rCD	S-rCD << HC	L-rCD < HC
	$P = 0.398$	$P = 0.064$	$P < 0.001$[a]	$P = 0.412$	$P = 0.001$[b]	$P = 0.021$[c]
038_Hippocampus_R	Right hippocampus	3.300 ± 0.313	3.336 ± 0.315	3.419 ± 0.239	3.508 ± 0.251	0.008[b]
Post hoc analysis	aCD = S-rCD	aCD = L-rCD	aCD <<< HC	S-rCD = L-rCD	S-rCD < HC	L-rCD = HC
	$P = 0.188$	$P = 0.063$	$P < 0.001$[a]	$P = 0.671$	$P = 0.034$[c]	$P = 0.105$
Type B: trends for enlargement (number of ROIs = 8)						
071_Caudate_L	Left caudate	3.470 ± 0.558	3.578 ± 0.556	3.929 ± 0.577	3.499 ± 0.545	0.002[b]
Post hoc analysis	aCD = S-rCD	aCD <<< L-rCD	aCD = HC	S-rCD < L-rCD	S-rCD = HC	L-rCD > HC
	$P = 0.173$	$P < 0.001$[a]	$P = 0.134$	$P = 0.043$[c]	$P = 0.858$	$P = 0.021$[c]
072_Caudate_R	Right caudate	3.615 ± 0.465	3.805 ± 0.460	3.988 ± 0.481	3.648 ± 0.479	0.002[b]
Post hoc analysis	aCD < S-rCD	aCD <<< L-rCD	aCD = HC	S-rCD = L-rCD	S-rCD = HC	L-rCD > HC
	$P = 0.024$[c]	$P < 0.001$[a]	$P = 0.089$	$P = 0.242$	$P = 0.351$	$P = 0.024$[c]
Type C: trends of irreversible changes (number of ROIs = 10)						
077_Thalamus_L	Left thalamus	3.908 ± 0.457	3.846 ± 0.460	4.028 ± 0.481	4.206 ± 0.479	0.001[b]
Post hoc analysis	aCD = S-rCD	aCD = L-rCD	aCD <<< HC	S-rCD = L-rCD	S-rCD <<< HC	L-rCD < HC
	$P = 0.931$	$P = 0.153$	$P < 0.001$[a]	$P = 0.206$	$P < 0.001$[a]	$P = 0.036$[c]
078_Thalamus_R	Right thalamus	4.013 ± 0.534	4.043 ± 0.512	4.295 ± 0.560	4.437 ± 0.552	<0.001[a]
Post hoc analysis	aCD = S-rCD	aCD < L-rCD	aCD <<< HC	S-rCD = L-rCD	S-rCD << HC	L-rCD = HC
	$P = 0.572$	$P = 0.012$[c]	$P < 0.001$[a]	$P = 0.105$	$P = 0.001$[b]	$P = 0.145$
Type D: trends of an uninfluenced (number of ROIs = 11)						
041_Amygdala_L	Left amygdala	1.050 ± 0.085	1.093 ± 0.085	1.075 ± 0.085	1.070 ± 0.089	0.139
Post hoc analysis	aCD << S-rCD	aCD = L-rCD	aCD <<< HC	S-rCD = L-rCD	S-rCD = HC	L-rCD = HC
	$P = 0.007$[b]	$P = 0.094$	$P = 0.006$[b]	$P = 0.354$	$P = 0.593$	$P = 0.587$
042_Amygdala_R	Right amygdala	1.137 ± 0.077	1.156 ± 0.074	1.158 ± 0.079	1.152 ± 0.081	0.511
Post hoc analysis	aCD = S-rCD	aCD = L-rCD	aCD <<< HC	S-rCD = L-rCD	S-rCD = HC	L-rCD = HC
	$P = 0.072$	$P = 0.109$	$P = 0.004$[b]	$P = 0.837$	$P = 0.638$	$P = 0.482$

Values are expressed as mean ± s.d. (cm^3). The *P* value among groups were obtained by one-way ANOVA and *post hoc* analysis.
[a]$P < 0.001$; [b]$P < 0.01$; [c]$P < 0.05$.
aCD, patient group with active CD; HC, healthy control group; L-rCD, patient group with long-term remitted CD; S-rCD, patient group with short-term remitted CD.

Type A trends toward normalization

In most brain subregions (87/116), the trends for GMV change after the resolution of hypercortisolism were similar to those of the total GMVs. The aCD group had the smallest GMV among the four groups, whereas the HC group had the largest GMV. The GMVs of the two remitted CD groups were between those of the aCD patients and HCs. These results indicated that trends for reversibility of regional GMVs also occurred after the resolution of hypercortisolism.

Moreover, in 83 subregions of type A, the GMVs were significantly or nearly significantly correlated with the remission time in the short-term-remitted CD group, indicating that these trends occurred in the short term following curative surgery. In some other subregions, such as the hippocampus, significant correlations between remission time and GMVs could only be found in the long-term-remitted CD group, indicating that the trends for recovery occurred in the late stage following curative surgery in these patients (Fig. 1, Types A1 and A2).

Type B trends for enlargement

In eight subregions (bilateral caudate, etc.), the GMVs of the two remitted CD groups were not only larger than those of the active CD patients but also larger than those of the HC subjects. No correlations with remission time were

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observed between the remitted CD groups. These results may indicate that, after the resolution of hypercortisolism, trends for increased GMVs might occur in these subregions (Fig. 1, Type B; Supplementary Table 3).

Type C trends of irreversible changes

In the bilateral thalamus and several subregions of the cerebellum, the GMVs of the HC group were significantly larger than those of any CD group. These results may indicate that after the resolution of hypercortisolism, no significant recovery of GMVs could be found in these regions. Thus, the reductions in GMVs in these regions seem to be irreversible (Fig. 1, Type C; Supplementary Table 3).

Type D trends of unaffected

In 11 subregions (bilateral amygdala, etc.), no significant difference in GMVs could be found among the four groups. In addition, no correlations were observed between remission time and GMVs in either of the remitted CD groups. These findings may indicate that neither hypercortisolism nor the resolution of hypercortisolism could influence these subregions (Fig. 1, Type D; Supplementary Table 3).

Preliminary longitudinal analysis

Patterns similar to those found *via* cross-sectional analysis could be found *via* one-way ANOVA. However, a significant and positive correlation with remission time was observed only in AAL 079 (Heschl left). The details of one-way ANOVA and correlation analysis of longitudinal data are presented in Supplementary Fig. 3.

Correlation analysis between GMVs and other clinical features in brain subregions

Negative correlations between disease duration and the GMVs of subregions were observed in the right operculum of the inferior frontal gyrus, bilateral triangle of the inferior frontal gyrus, right rectus, bilateral insula, left hippocampus, left middle occipital gyrus, left inferior occipital gyrus, bilateral postcentral gyrus, right inferior parietal gyrus, right supramarginal gyrus, bilateral angular gyrus, and left middle temple gyrus in long-term-remitted CD patients. Interestingly, in the bilateral caudate, positive correlations were observed between disease duration and GMV in CD patients with long-term remission, indicating that the duration of hypercortisolism exposure is related to the increase in GMV in the caudate. The results of correlated analysis with disease durations are presented in Supplementary Table 4. The GMVs of 49 subregions were found to be significantly correlated with the cortisol level in CD patients. The results of the correlation analysis with the level of cortisol are presented in Supplementary Table 5.

Discussion

Major finding

After the resolution of hypercortisolism, trends of possibly reversible alterations in GMV were observed in most brain subregions of CD patients. However, in other subregions, several different trends of GMV change were found. These results may indicate that, under chronic hypercortisolism exposure and after the resolution of the condition, the regional change in GMV in CD patients was a complex phenomenon, not mere partial reversibility.

Previous studies on hypercortisolism-related brain alterations

The limbic system plays an essential role in stress-related disorders, for example major depression and posttraumatic stress disorder (PTSD). It is rich in receptors for cortisol, especially mineralocorticoid receptors (MRs), to which both mineralocorticoids and glucocorticoids can bind (20). Experimental animal studies have already demonstrated the neurotoxic effects of hypercortisolism on the limbic system (21). Previous studies of CD patients have focused on alterations in the limbic system, especially in the hippocampus, associated with depression and anxiety (2, 6, 22, 23, 24). However, alteration of the limbic system cannot explain other factors, including the psychological problem of chronic exposure to hypercortisolism, cognitive impairment, and even dementia (25). Contrary to the limited distribution of MRs, glucocorticoid receptors (GRs), another type of cortisol receptor, are widely distributed throughout the brain (21). Glucocorticoids affect all cell types in the brain, including non-neuronal cells, such as microglia, oligodendrocytes, astrocytes, and endothelial cells (26). Several recent studies of CD patients demonstrated widely distributed alterations in structure, function, and blood supply as well as diffusion in the human brain (3, 17, 27). Thus, the widespread alterations observed in the current study are not only in line with those previous studies and our previous total GM study (12)

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but are also in line with the different patterns of regional morphological changes observed in the current study. This indicates that the influence of hypercortisolism on the human brain is a complex phenomenon.

Trends in GMV changes after the resolution of hypercortisolism

Compared with the GMVs of the HC group, significantly smaller GMVs were observed in most brain subregions of active CD patients. ANOVA analysis revealed trends of reversibility in these regions. In addition, significant and positive correlations were observed between GMVs in these subregions and remission time in short-term-remitted CD patients. Previous studies demonstrated not only partially reversible GMV reductions in particular brain subregions, such as the hippocampus (8, 28), but also similar partial reversibility in other brain subregions and in the whole brain (17, 29). Thus, it is reasonable to assume that partial reversibility of GMVs could be found, not only in particular brain subregions but also in most brain subregions of remitted CD patients.

Moreover, we found a significant and positive correlation between remission time and GMVs of the hippocampus and adjacent structures in CD patients with long-term remission. The hippocampus and adjacent regions are rich in GRs compared with the number of GRs in any other subregions of the human brain (21). Thus, these structures exhibit increased sensitivity to cortisol, affecting both volume loss and recovery (8, 17). Previous studies of stress-related disorders and neurodegenerative disorders have often demonstrated alterations in hippocampal volumes (30). However, our VBM study of short-term-remitted CD patients did not find any difference among active CD patients, short-term-remitted CD patients, and HCs in the hippocampus (11). Contrarily, significant alterations could be observed in the hippocampus among the three patient groups when compared with the hippocampus of HC subjects. Thus, as the hippocampus has an abundance of cortisol receptors, the trends of the hippocampal recovery process after hypercortisolism resolution might be quite different from the trends in other brain regions.

The GMVs in eight subregions (the bilateral supply motor area, bilateral caudate, bilateral paracentral lobule, and bilateral putamen) of the two remitted CD groups were not only larger than those of the active CD patients but also larger than those of the HC subjects. About 12 months after the curative operation, Starkman *et al.* found that an increase in the volume of the right caudate was significantly associated with decreases in depression and anxiety scores (9). However, without including any HC subjects, it was impossible to draw any further conclusions about whether caudate volume decreased in the active stage of CD. Contrarily, our previous VBM study found greater GMVs in the bilateral caudate of CD patients in short-term remission than in HC subjects (11). Moreover, we found a positive correlation between disease duration and GMVs in these regions of CD patients in long-term remission. According to a recently proposed theory, 'The Scaffolding Theory of Aging and Cognition', the process of neurodegeneration is characterized by both decline and preservation (31). This means that when reductions of GMV occurred in some brain regions, structural compensation could be observed in other regions (32, 33, 34). Nevertheless, it is likely that the cause of larger GMVs in these subregions is multifactorial, including intrinsic imperfections of surgery and endocrine replacement therapy. However, the possibility of a compensatory strategy after the resolution of hypercortisolism cannot be entirely neglected.

In the bilateral thalamus, the GMVs of the HC group were greater than those of any CD group, whereas no significant differences could be found between the active group and the two remitted CD groups. Irreversible effects on the human brain caused by chronic exposure to hypercortisolism have long been observed in CD patients with long-term remission (2). Our findings could indicate that the detriment induced by hypercortisolism might be irreversible in these regions.

The GMVs of several subregions, including the bilateral amygdala, demonstrated no difference among the four groups. Although previous studies revealed functional and structural alterations in the amygdala of adolescents with hypercortisolemia (14), no differences were found in amygdala activation of adult remitted CD patients (2). In structural MRI studies, Andela *et al.* did not find any volume difference in the bilateral amygdala (35). Santos *et al.* only found that the volumes of the right (but not the left) amygdala of active CS patients were barely smaller ($P=0.043$) than those of HCs (10). Our findings could indicate that hypercortisolism has at least some structural effect on some brain subregions.

Limitations

This analysis of altered GMVs in the brain subregions of CD patients has some limitations. Our study did not include neuropsychological tests or clinical data of HC subjects. These limitations and the cross-sectional

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design of our study prevent the determination of further conclusions with regard to alterations in regional GMVs caused by hypercortisolism, resolution of the condition, and influence of neuropsychological disorders on these effects. Thus, prospective functional MRI and structural MRI studies that include psychological measures and neuropsychological tests of CD patients and HC subjects are required.

Conclusion

Cross-sectional analysis revealed significantly altered GMVs and trends of reversibility in most brain subregions of CD patients. However, different trends of GMVs were observed in other brain subregions of these patients. These results indicated that regional morphological changes in the gray matter after the resolution of hypercortisolism in CD were due to complex phenomena. Our study enhances our understanding of the structural alterations in the human brain due to hypercortisolism (12). Furthermore, our study may facilitate the development of future studies on the influence of hypercortisolism, with comprehensive neuropsychological tests.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-21-0385.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author, but they are not publicly available due to privacy restrictions.

Author contribution statement

Hong Jiang and Wen-Jie Yang contributed equally to this work. Liu-Guan Bian and Chang Liu contributed equally to this work.

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