Gray matter abnormalities in pediatric focal and generalized epilepsy: A voxel-based morphometry study with MRI at 3.0 T

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Abstract

Background : Our aims were to explore gray matter volume (GMV) abnormalities in focal and generalized epilepsy children compared to healthy group using a voxel-based morphometry (VBM) methodology, and to determine whether the regions of any observed structural changes are correlated with clinical characteristics. **Methods:** Thirty-two epilepsy children (18 with focal epilepsy, 14 with generalized epilepsy) and 18 control subjects were recruited. All participants were imaged structural MRI scans with a 3.0 T MR system. VBM analysis using statistical parametric mapping 8 (SPM8) was first performed to detect gray matter reduction in focal and generalized epilepsy children compared to controls. Then we analyzed the correlations between GMV changes and clinical features of epilepsy patients. **Results:** In the patient-control group comparison of VBM, the left hippocampus, left thalamus, left paraHippocampal and bilateral putamen exhibited a significantly GMV decreased. In stratified analysis, the focal epilepsy children showed GMV reductions in left thalamus, left hippocampus and bilateral putamen relative to controls; the GMV exhibited extensive reductions in left parahippocampal, left putamen, and right supplementary motor area in generalized epilepsy children compared to controls. No significant difference was found in regions of GMV abnormalities correlated with disease duration and age of onset in both the focal and generalized epilepsy groups. **Conclusions:** By performing VBM to detect GMV changes in children, our study demonstrates the GMV reductions exist in different regions in focal and generalized epilepsy patients. Our study further provides structural neuroimaging evidence on the pathophysiology of focal and generalized epilepsy children.

Background

Epilepsy is a common pediatric problem, and of considerable challenge to pediatricians[1, 2]. Epilepsy can severely affect the development and function of immature brain, which may lead to cognitive or intellectual dysfunctions. The voxel-based morphometry (VBM), an automated technique for MRI analyses, is being increasingly applied in cerebral anatomical changes in epilepsy patients[3]. Through VBM emerged from recent developments in neuroimaging analysis techniques, investigators could explore gray matter volumes (GMV) abnormalities in epileptic patients to reveal the cause of the cognitive or intellectual dysfunctions.

Epilepsy can be divided into following diverse epilepsy types[4]: (1)focal epilepsy; (2)generalized epilepsy; (3)Combined Generalized and Focal Epilepsy; (4)Unknown. Different pathophysiological mechanisms between focal epilepsy and generalized epilepsy have been explored in previous studies[5-8]. The study about interictal excitability differences demonstrate the impact of cortical excitatory/inhibitory function manifest bilateral in idiopathic generalized epilepsy, whereas it diffuse over the epileptic focus but remained lateralized in focal epilepsy[8]. Although some endeavor have been made in this filed, the pathophysiological basis differences are not fully understood yet. In addition, there are less study using VBM to explore GMV abnormalities in focal and generalized epilepsy compared to control, especially within the scope of children.

Epilepsy is the most common neurological condition in children and the characteristics of high prevalence, high morbidity, and high costs can result in heavy burden on family and society[9]. The prevalence of epilepsy in children ranges from 3.2-5.5/1,000 in developed countries to 3.6-44/1,000 in underdeveloped countries[10]. The social problems deriving from children epilepsy may be worse in our country[11]. The prevalence of children lifetime epilepsy is 4.5‰ aged 0 to 4 years old, 5.34‰ aged 5 to 9 years old, 6.20‰ aged 10 to 14 years old in china[12]. The prevalence of children lifetime epilepsy gradually increases with age. There are a small number of studies concentrating on the patients under 3 years old, because previous studies demonstrated a significantly improved seizure outcome compared to rates in older cohorts[13-15]. Though the prevalence of children lifetime epilepsy in older children forms a large proportion, they get less focus. And it is more meaningful using older children as study population when exploring the relationship between age at seizure onset or epilepsy duration and changes in GMV.

Here, we investigated the regions of GMV abnormalities in focal and generalized epilepsy compared to the controls respectively. In addition, we correlated the volume changes of several brain regions with age at seizure onset and epilepsy duration. The aim of this study was to determine changes in GMV and associations with the development and progression of childhood epilepsy.

Methods

Subjects and clinical information

The study populations consisted of 32 right-handed epilepsy children aged 5 to 14 years old (mean age 8.6 ± 2.7 years, 24 males and 8 females). Epilepsy children were further divided into 18 right-handed children patients with focal epilepsy (mean age 8.6 ± 2.4 years, 14 males and 4 females) and 14 right-handed children patients with generalized epilepsy (mean age 8.6 ± 3.0 years, 10 males and 4 females). The diagnoses of epilepsy were determined in Guangzhou Women and Children's Medical center according to the criteria by the International League Against Epilepsy in 2017[4]. All patients had had MRI scans and had long-term EEG records. The inclusion criteria were (1) diagnosed by an Epileptologist based on clinical, EEG and MRI findings; (2) age between 5 and 14 years; (3) attendance at standard schools; (4) high-resolution MR images acquired on the same type 3T research scanner. The exclusion criteria were (1) existence of organic brain disorder; (2) abnormalities in brain MRI scans; (3) 'Combined Generalized and Focal Epilepsy' and 'Unknown' were not included.

For group comparison, 18 healthy children (mean age 8.3 ± 1.8 years, 11 males and 7 females) who were similar in age, gender and education level were recruited as control group. Children with IQ < 70, a past history of neurologic or psychiatric disorders, a family history of epilepsy or a history of usage of medications damaging nervous system were excluded. This study was approved by the local ethics committee and informed consent was obtained for all participants from their parents.

MRI Acquisition

All of the participants underwent high-resolution brain MRI on a Siemens Trio 3T scanner. For VBM analysis, high-resolution three-dimensional magnetization prepared rapid gradient echo (MPRAGE) sequence was acquired for each subject using the following parameters: TR = 1160ms, TE = 4.19ms, 3TI = 600ms, field of view = $140 \times 250\text{mm}^2$, matrix size = 256×192 , slice thickness = 1.2mm, and flip angle = 15°). During the process of scanning, all subjects were asked to be relaxed and keep their eyes closed but stay awake.

Voxel-based morphometry

The quantitative analysis of MRI was performed with the VBM8 Toolbox within SPM8 (Wellcome Trust Centre for Neuroimaging, <https://www.fil.ion.ucl.ac.uk/spm/>) running under MATLAB R2013b (The MathWorks, Natick, MA, USA). According the standard protocol, the VBM analysis comprised the following steps.

The quality of each image was inspected and the image origin was set to the anterior commissure. A customized template appropriate to the study population was created because of the wide range of age of participants. All native images were normalized to the same stereotactic space by registering each to the customized template image for spatial normalization. The normalized images were then segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). Finally, the normalized, segmented GM images were smoothed with an isotropic Gaussian kernel with a sigma of 8 mm for statistical analyses. Finally, differences in cerebral GM density between the patient and control groups were evaluated using the voxelwise generalised linear model applied using permutationbased non-parametric testing (5000 permutations).

Statistical analyses

Prior to statistical analyses, differences in demographic were identified using an independent two-sample t-test for continuous variables and χ^2 tests for dichotomous variables. The analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) and p values of <0.05 was considered indicative of statistical significance.

Using the general linear model, voxel-wise comparisons of GMV were performed between patients and controls, with age and gender as confounding covariates. The significance of group differences was estimated with the theory of $p < 0.001$ uncorrected and the cluster size was set at >100 voxels.

To investigate the relationship between duration of epilepsy or age at epilepsy onset and GMV, the general linear model was applied. A statistical significance was accepted if $p < 0.05$.

Results

Demographic and clinical characteristics

The details of demographic and clinical characteristics for patients and healthy groups are showed in Table 1. Age and gender did not differ between the control and local epilepsy patients, generalized epilepsy patients or total epilepsy patients group ($p>0.05$). Also the specific diagnosis of epileptic syndromes among focal epilepsy and generalized epilepsy groups are listed in Table 1.

VBM analysis between control group and total epilepsy patients

Three clusters exhibited significant decreases in GMV in the whole brain VBM comparison between patients with epilepsy and control group. Within these clusters, the left hippocampus, left thalamus, left paraHippocampal and bilateral putamen were involved (Table 2, Figure 1).

VBM analysis between control group and focal epilepsy, generalized epilepsy patients

Compared to controls, patients with local epilepsy had significant GMV reductions in left thalamus, left hippocampus and bilateral putamen ($p<0.001$, uncorrected, Table 3, Figure 2). In patients with generalized epilepsy, the GMV was significantly decreased in left parahippocampal, left putamen, and right supplementary motor area ($p<0.001$, uncorrected, Table 4, Figure 3).

Correlations with seizure onset age and epilepsy duration

In whole-brain voxel correlation analysis, no correlations were found between GMV and the duration of epilepsy, age at epilepsy onset in the focal epilepsy and generalized epilepsy group (Figure 4, Figure 5).

Discussion

The pathophysiologic and anatomical mechanism of two different types of epilepsy, focal epilepsy and generalized epilepsy, remains inconclusive. The application of VBM provides an relatively unbiased tool to study the structural abnormalities of whole brain grey matter [16]. Although many previous studies have investigate abnormalities in grey matter in patients with the specific epilepsy syndromes [17-21], few studies have examine GMV difference according to the classification of epilepsy type, focal and generalized epilepsy. To explore the serious effects of epilepsy on brain in incipient stage of life span, we used children as study objects.

This study attempted to explore the structural alterations of GMV in children with focal and generalized epilepsy, and to investigate the relationships between abnormal regions and clinical variables. Before classification by epilepsy type, compared to control group, GM abnormalities were demonstrated in the left hippocampus, left thalamus, left paraHippocampal and bilateral putamen showed. However, relative to healthy controls, extensive reductions of GMV were observed in left thalamus, left hippocampus and bilateral putamen in children with focal epilepsy. Also children with generalized epilepsy showed a significant GMV reduction in left parahippocampal, left putamen, and right supplementary motor area. Nevertheless, there were no significant relationships between seizure onset age, duration of epilepsy, and abnormal brain regions.

Up to now, many studies have focused on diverse epilepsy syndromes of focal epilepsy. The focal epilepsy of this study consist of BECTS, BCEOP, TLE, and FLE. In the early days of VBM applications, Keller first discussed hippocampal and extrahippocampal abnormalities in patients with temporal lobe epilepsy[22]. Then, Labate's VBM analysis provided evidence of a reduction in gray matter volume in the hippocampus and thalami[23]. Thereafter, increasing studies about temporal lobe epilepsy demonstrated widespread reductions of GMV in hippocampus and thalamus[24, 25]. In addition changes in hippocampus and thalami, a significant regional GMV atrophy was found involving the putamen, pallidum, middle and inferior temporal areas, amygdala and cerebellar hemisphere in patients with temporal lobe epilepsy[26]. However, in patients with BECTS, Yang found significant reductions in bilateral putamen, bilateral paracentral lobule and right supplementary motor area[27]. Here, we further demonstrated reductions of GMV in left thalamus, left hippocampus, and bilateral putamen in children with focal epilepsy. The abnormal regions may provided a mutual pathophysiologic and anatomical evidence among diverse epilepsy syndromes of focal epilepsy.

In the other part of our study, the generalized epilepsy is composed of patients with GTCS and CAE. A study recruiting 13 patients with CAE showed areas of GM decrease in both thalami and in the subcallosal gyrus[28]. It has also been demonstrated that the patients with CAE showed less GMV in the bilateral thalami in drug-naïve CAE[29]. However, our study showed decrease of GMV in left parahippocampal, left putamen, and right supplementary motor area. Tanji has reported the supplementary motor area (SMA) is involved with global tonic seizure[30], and neural fasciculus emitted from SMA area connects putamen, thalami, pons and spinal. Otherwise, reduction of GMV in thalami was not be found in this study, which may be on account of Insufficient sample size or a mixture of different epilepsy syndromes. Nevertheless, simultaneous reductions in several regions in our study may provide new ideas for studying specific mechanisms of generalized epilepsy.

In summary, we further evaluated clinical variables and relationships with respect to GMV demonstrated by optimized VBM analysis in focal epilepsy and generalized epilepsy patients. However, there was no significant difference in correlation analysis.

Conclusions

Our study reveals there are different regions of GMV reductions in focal and generalized epilepsy patients using the VBM method in SPM8 plus the DARTEL algorithm and a novel segmentation toolbox. Our study further provides structural neuroimaging evidence on the diagnosis and the pathophysiology of focal and generalized epilepsy children.

Abbreviations

GMV: Gray matter volume; VBM: Voxel-based morphometry; SPM8: Statistical parametric mapping 8; MPAGE: Magnetization prepared rapid gradient echo ; GM: Gray matter; WM: White matter; CSF: Cerebrospinal fluid; BECTS: Benign epilepsy with centro-temporal spikes; BCEOP: Benign childhood

epilepsy with occipital paroxysm; FLE: Frontal lobe epilepsy; TLE: Temporal lobe epilepsy; GTCS: IGE with generalized tonic clonic seizures only; CAE: Childhood absence epilepsy.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee and Institutional Review Board of Guangzhou Women's and Children's Medical Center, Guangzhou, China, and conducted in accordance with the ethical guidelines of the Declaration of Helsinki of the World Medical Association (IRB No. 2019-30000). Written informed consent was

obtained from all individual participants included in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analyzed during the current study are 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

HRZ: draft of manuscript, including description of study, results, analysis and the interpretation of data. HSL: data acquisition, protocol design (MRI) and acquisition of demographical information of participants (EEG, seizure type). WXC: clinical interpretation of results and final approval of the manuscript. GJL: statistical analysis, interpretation of data and final approval of the manuscript. BSZ: critical revision of manuscript for important intellectual content and final approval of the manuscript. HYL: study design and supervision, revision of manuscript for content and obtaining funding. All authors read and approved the final manuscript.

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- ## Tables

Clinical parameter	Epilepsy group			Control group	P ₁	P ₂	P ₃
	Focal	Generalized	Total				
	epilepsy	epilepsy					
Number of subjects	18	14	32	18			
Age(years)	8.6± 2.4	8.6± 3.0	8.6± 2.7	8.2± 1.7	0.639	0.624	0.598
Gender(F:M)	14:4	10:4	24:8	11:7	0.278	0.542	0.304
Seizure onset age	7.8±2.5	6.3±3.5	7.1±3.0				
Duration of epilepsy	1.1±0.9	2.5±2.4	1.7±1.9				
Epileptic syndromes	BECTS 7 BCEOP4 TLE 4 FLE 3	GTCS 9 CAE 5					

P₁:Focal epilepsy patients versus Control group; P₂: Generalized epilepsy patients versus Control group; P₃: Total epilepsy patients versus Control group. BECTS: benign epilepsy with centro-temporal spikes, BCEOP: benign childhood epilepsy with occipital paroxysm, FLE: frontal lobe epilepsy, TLE: temporal lobe epilepsy, GTCS: IGE with generalized tonic clonic seizures only, CAE: childhood absence epilepsy.

Table 2. Brain regions with decreased GMV between control and total epilepsy patients

Anatomical localization	Side	Cluster size	MNI coordinate areas			T-score
			X	Y	Z	
44% Hippocampus	L	786	0	-36	-9	5.0384
36% Thalamus	L					
46% Putamen	L	489	-30	-1.5	-7.5	7.2036
18%ParaHippocampal	L					
85%Putamen	R	183	30	-12	-1.5	5.1587

Cluster size: cluster extent threshold (=100 voxels), L: left, R: right, MNI: Montreal Neurological Institute,T-score: Two sample t-paired test, uncorrected, minimum of 100 voxels.

Table 3. GM abnormalities in patients with focal epilepsy compared to controls.

Anatomical localization	Side	Cluster size	MNI coordinate areas			T-score
			X	Y	Z	
70% Putamen	L	307	-30	-1.5	-7.5	6.4599
54% Thalamus	L	787	-9	-33	9	4.9535
24% Hippocampus	L					
84% Putamen	R	200	31.5	-10.5	-17.9	4.9661

Cluster size: cluster extent threshold (=100 voxels), L: left, R: right, MNI: Montreal Neurological Institute,T-score: Two sample t-paired test, uncorrected, minimum of 100 voxels.

Table 4. GM abnormalities in patients with generalized epilepsy compared to controls.

Anatomical localization	Side	Cluster size	MNI coordinate areas			T-score
			X	Y	Z	
63% ParaHippocampal	L	184	-16.5	1.5	-22.5	5.9154
98% Putamen	L	361	-28.5	-6.3	-7.5	6.162
88% Supp_Motor_Area	R	132	3	-22.5	54	4.1133

Cluster size: cluster extent threshold (=100 voxels), L: left, R: right, MNI: Montreal Neurological Institute,T-score: Two sample t-paired test, uncorrected, minimum of 100 voxels.

Figures

Figure 1

Grey matter abnormalities in epilepsy patients: cluster level significant regions(peak level $p < 0.001$ and cluster extent threshold =100 voxels). Left thalamus, left hippocampus, left ParaHippocampal and bilateral putamen were observed relatively decreased.

Figure 2

Grey matter abnormalities in focal epilepsy: cluster level significant regions(peak level $p < 0.001$ and cluster extent threshold =100 voxels). Abnormalities appear to be relatively decreased in left thalamus, left hippocampus and bilateral putamen.

Figure 3

Grey matter abnormalities in generalizes epilepsy: cluster level significant regions (peak level $p < 0.001$ and cluster extent threshold =100 voxels). Abnormalities appear to be relatively decreased in left parahippocampal, left putamen, and right supplementary motor area.

Figure 4

Relationship between GMV and the duration of epilepsy, age at epilepsy onset in the focal epilepsy.

Figure 5

Relationship between GMV and the duration of epilepsy, age at epilepsy onset in the generalized epilepsy.