Short Communication

Brainstem dysfunction in patients with late-onset Lennox–Gastaut syndrome: Voxel-based morphometry and tract-based spatial statistics study

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Abstract

Background: There have been a few reports of patients who developed Lennox–Gastaut syndrome (LGS) in the second decades of their life. **Objectives:** The aim of this study was to investigate electroclinical presentation in patients with late-onset LGS. In addition, we evaluated structural abnormalities of the brain, which may give some clue about the common pathogenic pathway in LGS. **Materials and Methods:** We enrolled the patients with late-onset LGS. We collected electroclinical characteristics of the patients and evaluated structural abnormalities using voxel-based morphometry (VBM) and tract-based spatial statistics (TBSS) analysis. **Results:** The three subjects were diagnosed with late-onset LGS. The patients have no mental retardation and normal background activities on electroencephalography (EEG), and they had generalized paroxysmal fast activities on EEG, especially during sleep. The TBSS analysis revealed that fractional anisotropy values in the patients were significantly reduced in the white matter of brainstem compared with normal controls. However, VBM analysis did not show any significant difference between the patients and normal controls. **Conclusions:** Patients with late-onset LGS have different clinical and EEG characteristics from those with early-onset LGS. In addition, we demonstrated that brainstem dysfunction might contribute to the pathogenesis of late-onset LGS.

Key Words

Brain stem, Lennox–Gastaut syndrome, magnetic resonance imaging

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Introduction

Lennox–Gastaut syndrome (LGS) is a severe epileptic encephalopathy.[1] The triad of LGS consists of intractable seizures such as primarily tonic, atonic, and atypical absence seizures; generalized slow spike-waves (SSWs) (<2.5 Hz) and generalized paroxysmal fast activities (GPFAs) on electroencephalography (EEG); and cognitive dysfunction.[1] The majority of LGS have onset before the age of 8 years, with a peak onset age between 3 and 5 years.[1] However, there have been a few reports of patients who developed LGS in the second decades of their life with better cognitive function.[2,3]

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The etiology of LGS has been divided into two groups.[4] Approximately, 75% of cases are thought to be symptomatic, implying an identifiable cause such as cortical malformation or hypoxic damage. The cryptogenic group may account for approximately 25% of cases, in which a genetic predisposition and autoimmune influence have been hypothesized.[4] The electroclinical presentation of LGS is remarkably similar despite

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of a wide variety of underlying causes, including lesions in varying locations, suggesting that a common pathogenic pathway may be involved. However, the common pathogenic pathway in LGS has not yet been identified.

Recently, magnetic resonance imaging (MRI) has emerged as a reliable measure to discover the pathogenic pathway in various neurological disorders. On the one hand, MRI-based measures of gray matter atrophy are regarded as valid markers of disease state and progression of neurological disorder.[5] Voxel-based morphometry (VBM) was designed to increase sensitivity comparing the local composition of different brain tissue types with the discounting of positional and other large-scale volumetric differences in gross anatomy. On the other hand, the abnormal function of the brain in neurological disorder can be explained injured white matter fibers, which is typically observed by diffusion tensor imaging (DTI).[6] DTI can be used to reveal the injured regions of white matter, by examining the diffusion movement of water molecules within the white matter fiber bundles.[6] In particular, tract-based spatial statistics (TBSS) can improve the statistic power of multi-subject study of DTI data.[7] However, no studies have been conducted with VBM or TBSS in patients with LGS.

We studied three patients with LGS having onset in the second decades of their life, and they had normal routine MRI with visual inspection. The aim of this study was to investigate electroclinical presentation in patients with late-onset LGS. In addition, we evaluated structural abnormalities using VBM and TBSS analysis, which may give some clue about the common pathogenic pathway in LGS.

Materials and Methods

Subjects

This study was conducted with the approval of Institutional Review Board. This study was consecutively performed in a single tertiary hospital. Since March 2010, we enrolled three patients with a clinical diagnosis of late-onset LGS.[1,4] All of the patients had (1) the presence of multiple seizure types, (2) drug resistance to treatment with antiepileptic drugs (AEDs), (3) characteristic EEG abnormalities including GPFA and/or SSWs, and (4) age of seizure onset >8 years. We collected demographic and clinical characteristics including age, sex, occupations, age of seizure onset, epilepsy duration, and number of AEDs from these patients at the time of the MRI. In addition, all of the patients were evaluated with video-EEG monitoring.

The control group for quantitative MRI analysis consisted of five healthy age- and sex-matched subjects. All subjects had a normal neurological examination and no significant medical history. All control subjects had normal MRIs with visual inspection.

Magnetic resonance imaging data acquisition

All scans were performed on a 3.0T MRI scanner (AchievaTx, Phillips Healthcare, Best, The Netherlands) equipped with an 8-channel head coil. The three-dimensional (3D) T1-weighted images were obtained with a turbo field echo sequence with the following parameters: TI = 1300 ms, TR/TE = 8.6/3.96 ms, flip angle = 8°, and a 1 mm^3 isotropic voxel size. Sagittal-oriented high-resolution contiguous 3D T1-weighted images were obtained. To speed-up data acquisition, sensitivity encoding parallel imaging with an acceleration factor of two was applied. DTI was performed using spin-echo single shot echo-planar pulse sequence with a total of 32 different diffusion directions (TR/TE = 8620/85 ms, FA = 90°, slice thickness = 2.25 mm, acquisition matrix = 120 × 120, FOV = 240 × 240 mm^2, and *b*-value = 1000 s/mm^2).

Voxel-based morphometry and tract-based spatial statistics analysis

VBM based on 3D T1-weighted images was utilized to analyze volumetric difference. Image processing was performed using the VBM 8 toolbox (http://dbm.neuro.unijena.de/vbm), implemented in Statistical Parametric Mapping 8 (SPM 8, UK, http://www.fil.ion.ucl.ac.uk/spm). The VBM data were processed in the standard manner including the following steps: spatial normalization to the Montreal Neurological Institute (MNI, Montreal, Canada) template, gray matter segmentation, intensity modulation using Jacobian determination, and spatial smoothing with 6 mm Gaussian kernel. For the group analysis, two sample *t*-tests (patients vs. normal controls) were performed using a general linear model to identify whole brain gray matter alterations with significant differences. Significance was defined at the level of $P < 0.05$ after correcting for the family-wise error rate (FWE) to compensate for type 1 errors and applying an extent threshold of 30 voxels. To consider other effects, such as age, gender, and total intracranial volume; the two sample *t*-tests were repeated once with these factors, which were entered as covariates.

To perform TBSS analysis, all raw DTI data were preprocessed with FSL (http://www.fmrib.ox.au.uk/fsl). First, eddy current distortions and head motions were corrected by spatially normalizing all the diffusion weighted images. Subsequently, skull-stripping was applied to exclude nonbrain tissues and regions. Finally, we computed the diffusion tensor as well as scalar measures, including fractional anisotropy (FA) and mean diffusivity (MD). FA and MD were analyzed with protocols provided by TBSS.[7] We normalized individual FA volumes of the two groups to the MNI template space via affine registration. The aligned FA images were averaged to yield a mean FA image and then thinned to create the FA skeleton of the mean FA image. The skeleton was regarded to represent the common tract pattern of all participants from the two groups. Then, the FA threshold (0.2) was set on the skeleton to exclude gray matter and cerebral spinal fluid from the final analysis. Each subject's FA image was projected on to the skeleton. The significance threshold for between group differences was set at $P < 0.05$ (FWE corrected for multiple comparisons) using the threshold-free cluster enhancement option in the "randomize" permutation-testing tool in FSL (5000 permutations). Regional FA differences were then localized according to the probabilistic Johns Hopkins University, White Matter Atlas. Similarly, group comparisons of MD images were performed, respectively.

Results

Clinical, electroencephalography, and routine magnetic resonance imaging findings

The main clinical and EEG findings are summarized in Table 1. The mean age of seizure onset was 14 years (range

12–17 years), and the mean age at the time of MRI was 28 years (range 22–46 years). Of the three patients, two patients were students, and the other was an office worker. All of the patients live with normal daily activities. None of the cases had West syndrome preceded the onset of LGS. All of the patients had multiple types of seizures, such as tonic, atypical absence, generalized tonic-clonic, and focal seizures. Especially, all of the patients commonly had tonic seizures. All of the patients had intractable epilepsy despite of polypharmacy with AEDs.

All of the patients had normal background activities [Figure 1a], but they had interictal epileptiform discharges on EEG. The EEG showed GPFA during sleep in all of the three patients, whereas no patient had GPFA in wakeful state [Figure 1b]. SSWs were also seen in all of the three patients on EEG, which were observed during sleep and wakeful states. In addition, two of the patients had multifocal sharp waves on EEG.

All patients had normal brain MRIs on inspection.

Voxel-based morphometry and tract-based spatial statistics analysis results

TBSS analysis revealed that FA values in the patients were significantly reduced in the white matter of brainstem, especially in a pons, compared with normal controls (voxels = 129,193, peak voxel coordinate = −3, −37, −43, anatomic regions = left medial lemniscus) [Figure 2a]. In addition, increase of MD was also exhibited in the white matter of brainstem (voxels = 128,188, peak voxel coordinate = 10, −43, −39, anatomic regions = right inferior cerebellar peduncle) [Figure 2b].

However, VBM analysis did not show any significant difference between the patients and normal controls.

Discussion

We found that patients with late-onset LGS have no mental retardation and normal background activities on EEG, and they had GPFA on EEG, especially during sleep, which suggested that an overnight EEG study might be helpful for diagnosis of late-onset LGS. In addition, we demonstrated white matter changes of the brainstem in the patients with late-onset LGS using TBSS analysis. This finding suggested that brainstem dysfunction might contribute to the pathogenesis of late-onset LGS.

Our patients had late-onset of seizures, without mental retardation, and normal background activities on EEG; these findings are atypical for LGS.[1] However, the patients showed multiple seizure types that were intractable to AEDs and GPFA/SSWs on EEG, leading to a diagnosis of LGS. GPFA and tonic seizures are the most characteristic features of LGS, and these characteristics help distinguish LGS from the other epileptic encephalopathies.[2] Tonic seizures are characterized by a diffuse high-voltage slow wave followed by generalized low-voltage fast activity, which were similar electrical features of GPFA, suggesting that they probably recruit similar brain

Table 1: The clinical and electroencephalographic characteristics of the patients

	Patient 1	Patient 2	Patient 3
Age	22	17	46
Sex	Male	Male	Male
Age of seizure onset	14	12	17
Seizure types	Tonic, atypical absence, GTC	Tonic, atypical absence, focal	Tonic, focal
Mental retardation	None	None	None
Neurologic exam	Normal	Normal	Normal
Background activity on EEG	Normal	Normal	Normal
Epileptiform discharges on EEG	GPFA, SSWs	GPFA, SSWs, Multifocal sharp waves on bilateral frontal, temporal, and occipital lobes	GPFA, SSWs, multifocal sharp waves on bilateral frontal lobes
Brain MRI	Normal	Normal	Normal
AEDs	Valporic acid, zonisamide	Lamotrigine, valproic acid, levetiracetam, topiramate	Carbamazepine, clobazam

GTC = Generalized tonic-clonic, EEG = Electroencephalography, GPFA = Generalized paroxysmal fast activities, SSWs = Slow spike-waves, MRI = Magnetic resonance imaging, AEDs = Antiepileptic drugs

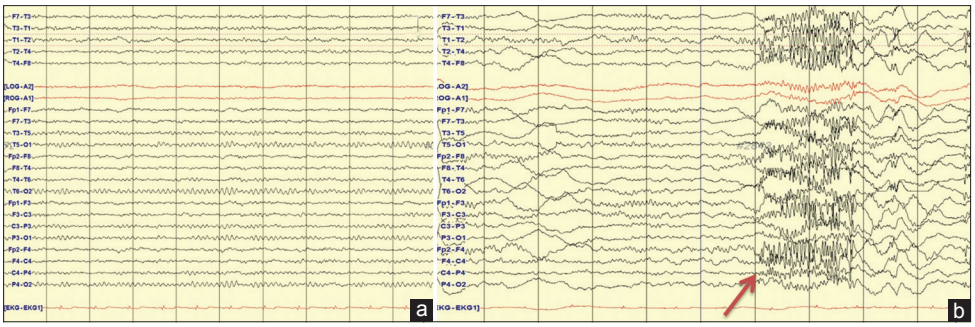

Figure 1: The electroencephalography a patient with late-onset Lennox–Gastaut syndrome. It reveals normal background activities at 9 Hz on awaken state (a) and shows generalized paroxysmal fast activities at approximately 15–20 Hz during sleep (b) (arrow)

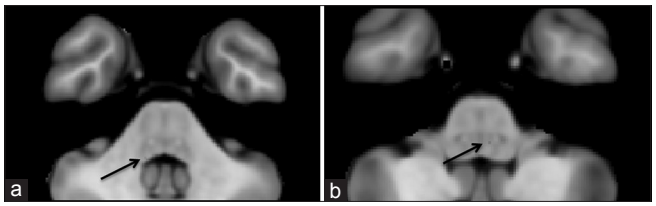

Figure 2: Tract-based spatial statistics result of fractional anisotropy images and mean diffusivity images. Red and yellow represents regions with decreased fractional anisotropy (a) and increased mean diffusivity (b) in patients compared to normal controls (arrows)

networks.[8] Our three patients had tonic seizures, and we recorded them by video-EEG monitoring. The ictal EEG of tonic seizures showed generalized low-voltage rhythmic fast activities at 10–20 Hz, which is compatible with tonic seizures in LGS.[1,2] Although paroxysmal fast activities are not pathognomonic for LGS because these activities may also be observed in focal lesional epilepsy, paroxysmal fast activities are suggestive of LGS, especially with bilateral GPFA.[2,9] All of our patients had GPFA. Interestingly, GPFA were especially observed during sleep, which was consistent with the previous study. The study investigated the evolution pattern of characteristics in patients with LGS during long-term follow-up and revealed that the evolution of EEG findings was characterized by the normalization of background activities in 44% of patients and a reduction of epileptiform discharges during wakefulness in 74% of patients in adulthood. Conversely, GPFA during sleep were retained in all patients in adulthood.[10] Thus, the authors suggested that overnight EEG was needed to evaluate adult LGS patients, and our cases also supported it.[10]

LGS typically begins during childhood and persists through adolescence and into adulthood.[11] The typical characteristics of early-onset LSG may change over time, and patients display very different clinical and EEG features when they reach adulthood.[11] The number and variety of seizure types usually decrease over time, despite persisting tonic seizures, particularly during sleep, and only a minority of patients display SSW complexes on EEG.[11] On the other hand, LGS can rarely be developed at adolescence like our cases, with less pronounced cognitive dysfunction.[11] This pattern reflects that seizures starting from late-onset may have a less damaging effect on cognitive development in late-onset LGS because the brain has already developed beyond critical developmental stages, such as synaptogenesis and apoptosis.[11] The previous studies have suggested that abnormal background activities on EEG were associated with underlying diffuse structural brain injury and cognitive dysfunction.[12,13] Alternatively, this finding may be explained by the theory of intrinsic disease severity.[14] This theory confers that the nature of the underlying intrinsic disease severity is variable, despite of same disease, and the disease may be defined as a complex interaction among underlying pathologies, individual genetics, and the environment.[14] A previous our study suggested that a biomarker for intrinsic disease severity in epilepsy was the age of seizure onset, and patients with early-onset epilepsy may have more severe intrinsic disease severity than those with late-onset epilepsy.[15,16] This theory can be applied to LGS. Patients with late-onset LGS may have less severe intrinsic disease severity; thus, they had no mental retardation and normal background activities on EEG. To confirm our hypothesis, an evaluation of the demographic and clinical differences between patients with early-onset and late-onset LGS may be needed.

Interestingly, we found the white matter changes of the brainstem in the patients with late-onset LGS, which suggested that brainstem dysfunction might contribute to the pathogenesis of late-onset LGS. A recent study also supports our finding; atrophy maximal in the pons and cerebellum mimics the patterns of seizure spread that have been previously observed during tonic seizures.[17] They also showed that gray matter atrophy was apparent in the mesial frontal lobe suggesting this region may be an important node in the epilepsy network of LGS. However, our study failed to demonstrate reduced volume in the cortex, which could be explained by late-onset LGS in our cases. This finding might be consistent with the clinical and EEG characteristics of the patients with late-onset LGS, who have tonic seizures and GPFA on EEG. This assumption is supported by several evidences. First, the tonics seizures and GPFA are usually bilateral and/or synchronous, which suggest that subcortical structures such as brainstem can be implicated in epileptogenesis of late-onset LGS. A recent study using simultaneous measurement of EEG-resting state function, MRI demonstrated that GPFA showed almost uniform increase in blood oxygen level-dependent signal in subcortical structures including brainstem as well as cortical areas.[18] In addition, tonic seizures have been observed in a hydranencephalic patient, and tonic seizures fail to respond to callosotomy.[19,20] Second, a pathologic study demonstrated the reduced expression of tyrosine hydroxylase, methionine encephalin, and parvalbumin in the brainstem in patients with LGS.[21] Third, there was early hyperperfusion in brainstem as well as cortical areas in a single photon emission computerized tomography study of the seizures of LGS.[22] They postulated that tonic seizures recruited the cortico-reticular system, which connected frontal areas to the pontine reticular formation, and was normally responsible for the postural tone and orienting behavior. Taken all together, based on the previous studies with our present finding of brainstem dysfunction in late-onset LGS, we can infer that the brainstem dysfunction influences the cerebral cortex through cortical projection to bring out characteristic tonic seizures and GPFA on EEG in patients with late-onset LGS, acting as a synchronizer, and amplifier rather than initiator of seizures.[8]

Conclusions

Our three cases suggested that patients with late-onset LGS have different clinical and EEG characteristics from those with early-onset LGS, and an overnight EEG study may help diagnose late-onset LGS by finding GPFA on EEG. In addition, we demonstrated that brainstem dysfunction might contribute to the pathogenesis of late-onset LGS.

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Conflicts of interest

There are no conflicts of interest.

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