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Gray matter alteration in patients with restless legs syndrome: a voxel-based morphometry study

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ABSTRACT

The purpose of this study was to demonstrate whether or not restless legs syndrome (RLS) is associated with any morphological change in gray matter. Forty-six RLS subjects and 46 controls were enrolled. We performed voxel-based morphometry analysis and compared the results of the two groups. The RLS subjects showed significant regional decreases of gray matter volume in the left hippocampal gyrus, both parietal lobes, medial frontal areas and cerebellum (uncorrected, $P<.001$). We found that RLS patients showed structural alteration in the brain and alterations in certain parts of the brain in RLS patients are relevant to RLS.

1. Introduction

Restless legs syndrome (RLS) is a sensorimotor neurological disorder, characterized by an urge to move the legs that is intimately associated with an uncomfortable sensation in the leg [1]. Although iron insufficiency and dopamine have key roles in the pathophysiology of this disease, substantially less is known about the neuroanatomical basis of this disease [2]. Also, it is known that RLS is a central nerve system, mainly brain, disorder. As a brain disorder, structural brain imaging studies would be interesting, but prior studies of structural brain images in RLS patients, using voxel-based morphometry (VBM) and diffusion tensor imaging, have reported contrasting results [3–10]. Some studies showed no specific brain alteration, and others showed some structural brain alterations (Table 1). Obviously, these discrepancies might be explained by methodological differences between the studies. However, these methodological differences alone might not be sufficient to explain the different results between the studies. The heterogeneity of RLS might also be reflected in the differences between the studies. Recently, a study showed that postmortem RLS brains have widespread impairment in myelin [5]. These postmortem areas were also those that demonstrated reduced volume with voxel-based analyses and were consistent with that seen with experimentally induced brain iron deficiency [5]. Thus, there is supported evidence that some structural alteration exists in the brain of RLS patients, and it may be related with the pathophysiology of RLS. However, as previous studies had contrasting results and there had been no such study in the Asian RLS population, we decided to do this study. Mainly, we are learning about the understanding of the pathophysiological mechanism of RLS. In addition there is no biomarker to diagnosis RLS, so far to the authors' knowledge. Therefore, the VBM study would be valuable in understanding the pathophysiology, particularly the neuroanatomical basis of this disease.

We have speculated that some structural brain alternation does exist in patients with RLS. The purpose of our study is to demonstrate whether or not RLS is associated with any brain alteration using VBM and if any clinical variables influence these alterations.

2. Materials and methods

2.1. Study subjects

This study recruited 46 subjects above the age of 18 who were diagnosed as primary RLS and who visited a University Hospital Outpatient Sleep Disorders Center. We enrolled all consecutive RLS patients who consented to participate in this study and 46 age- and gender-matched healthy controls. The healthy controls were selected after screening for any sleep disorders including RLS or serious

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Table 1
Summary of the brain volumetric studies in patients with RLS

Year, author	Magnet strength	TR/TE voxel size	No. of patients/control	Analysis	Results
2005, Etgen et al.	1.5 T	11.08/4 1×1×1.08	51/51	SPM1 software	GM increase in pulvinar bilaterally (statistical test: GLM; threshold at $P<.001$ uncorrected)
2007, Hornyak et al.	1.5 T	1×1×1	14/14	SPM2 software	GM increase in left hippocampus and middle orbitofrontal gyrus (statistical test: ANCOVA; threshold at $P<.05$ corrected for multiple comparisons)
2007, Unrath et al.	1.5 T	9.7/3.93 1×1×1	63/40	SPM2 software	GM decreased in the bihemispheric primary somatosensory cortex, which additionally extended into left-sided primary motor areas (statistical test: ANCOVA; threshold at $P<.05$ corrected for small-volume. No significant results at $P<.05$ corrected for multiple comparisons)
2010, Celle et al.	1.5 T	1900/3.95 2×2×2	17/54	SPM2 software	No significant results at $P<.05$ corrected for multiple comparisons. Statistical test: unpaired Student's t test (GM increase in left inferior occipital and left calcarine cortex and GM decrease in right superior temporal cortex at $P<.001$ uncorrected).
2011, Connor et al.	3 T	9.87/4.59 1×1×1	23/23	SPM5 software	Reductions in WM volumes in small areas of the genu of the corpus callosum, anterior cingulum and precentral gyrus (statistical test: paired Student's t test; threshold at $P<.001$ uncorrected)
2012, Comley et al.	1.5 T	20/5 1.02×1.02×1	16/16	FSL-VBM software	No significant results at $P<.05$ corrected for multiple comparisons. Statistical test: unpaired Student's t test
2012, Margariti et al.	1.5 T	25/4.6 0.86×0.86×1	11/11	SPM5 Software	No significant results at $P<.05$ corrected for multiple comparisons
2012, Rizzo et al.	1.5 T	5.1/12.5	20/20	SPM8 and FSL-VBM software	No significant results in all analysis at $P<.05$ corrected for multiple comparisons
This study	3 T	6/2.2	46/46	VBM using SPM8 VBM8 toolbox	Reduced volume in some areas of the left hippocampal gyrus, both parietal lobes, medial frontal areas, lateral temporal areas and cerebellum (uncorrected, $P<.001$)

ANCOVA, analysis of covariance.

medical disorders. The diagnosis was based on diagnostic standards set by the National Institutes of Health workshop on RLS [1] and was made during face-to-face interviews utilizing the validated Korean-language version [11] of the John Hopkins Telephone diagnostic questionnaire [12]. We excluded the RLS mimics during face-to-face interviews and physical examinations and laboratory tests in person. All subjects were diagnosed by a sleep specialist (C.Y.W.) who is an expert in RLS. We also excluded comorbidities to RLS or secondary RLS caused by pregnancy or by other diseases such as chronic kidney disease or peripheral neuropathy. However, subjects with only peripheral iron deficiency without definite cause were included. We also excluded other comorbid sleep disorders such as primary insomnia, sleep disordered breathing, circadian sleep disorders and parasomnia through analysis of the sleep questionnaires. The questionnaires used in this study underwent a validation process in Korean populations [13–15]. The severity of RLS symptoms was evaluated from the validated Korean-language version [16] of the International RLS scale (K-IRLS) [17]. All RLS subjects had moderate to severe RLS symptoms (K-IRLS≥15).

2.2. MR image acquisition and analysis methods

Magnetic resonance imaging (MRI) was performed on a GE VHi scanner operating at 3.0T (GE Medical Systems, Milwaukee, WI, USA). A three-dimensional (3D) anatomical MRI was obtained on each subject using a T1-weighted 3D spoiled gradient recalled (3D-SPGR) sequence (repetition time (TR)=6 ms, echo time (TE)=2.2 ms, flip angle=20°, field of view=240 mm, 256×256, 152 axial slices, slice thickness=2 mm thick). A T1-weighted 3D-SPGR sequence was employed in the current study because this sequence provides high resolution and good contrast between gray and white matter (WM) structures for a VBM.

All 3D-SPGR data were processed in the same method using a VBM toolbox version 8.0 (http://dbm.neuro.uni-jena.de) with SPM8 (Institute of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Tissue probability maps [TPM; gray matter (GM), WM and cerebrospinal fluid (CSF)] were created for optimizing registration with obtained T1-weighted images using 126 normal Korean male subjects' T1 anatomical data (mean age±S.D.: 34.25±11.14). The Template-O-Matic toolbox (http://irc.cchmc.org/software/tom.php) was used to create a template of TPM.

Images were corrected for a bias field in homogeneities and registered using linear 12-parameter affine and nonlinear transforms, and tissue was classified into GM, WM and CSF within the same generative model [18]. The segmentation procedure was performed by DARTEL for high-dimensional warping [19] by accounting for partial volume effect [20], by applying adaptive maximum a posterior estimations [21] and by a hidden Markov random filed model [22]. The GM images were modulated to account for volume changes resulting from the normalization process with an in house created TPM template. Finally, images were smoothed with a Gaussian kernel of 8-mm isolation window (full width at half maximum). Image analyses were made and data recorded by a single rater blinded to the clinical diagnosis of the patient.

The study was approved by the institutional ethics committee of a regional hospital. Informed consent was obtained from all participating subjects.

2.3. Statistical analysis

The general linear model (GLM) testing was used to find any structural changes of GM throughout the whole brain. A whole brain-based statistical approach instead of a region of interest (ROI)-based method was chosen since this type of approach is free from any a

Table 2
Demographic and clinical characteristics

Characteristics	RLS ($n=46$)	Controls ($n=46$)	p
Age (years)	55.9±11.4	53.0±11.9	.278
Gender, male (%)/female (%)	14 (30.4)/32 (69.6)	18 (39.1)/28 (60.9)	.381
RLS severity (K-IRLS)	26.9±7.0		
Symptom duration (months)	123.9±101.4		
Onset type (early/late)	21 (45.7%)/25 (54.3%)		
Serum ferritin	53.13±50.07		
Medications			
Yes (dopamine agonists)	13 (28.3%)		
No	33 (71.7%)		