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Alterations of mean diffusivity of pedunculopontine nucleus pathway in Parkinson's disease patients with freezing of gait

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ABSTRACT

Background: Although freezing of gait (FOG) is a common and disabling symptom in Parkinson's disease (PD), the underlying mechanism of FOG has not been clearly elucidated. Using analysis of diffusion tensor imaging (DTI), we investigated anatomic structures associated with FOG in PD patients.
Methods: We enrolled 33 controls and 42 PD patients (19 patients with FOG and 23 without FOG). DTI data were compared between PD patients and controls, and also between PD patients with and without FOG. Whole brain voxel-based analysis and regions of interest analysis in the pedunculopontine nucleus were used for DTI analysis.
Results: Compared with normal controls, PD patients showed microstructural changes in various subcortical structures (substantia nigra, globus pallidum and thalamus), frontal and insula cortex. PD patients with FOG demonstrated altered mean diffusivities in subcortical structures connected with pedunculopontine nucleus, such as basal ganglia, thalamus and cerebellum in voxel-based analysis. Using region of interest analysis of pedunculopontine nucleus, fractional anisotropy values were reduced and mean diffusivity values were increased bilaterally in PD patients with FOG. In correlation analysis, the fractional anisotropy value of the right pedunculopontine nucleus was moderately correlated with the severity of FOG.
Conclusions: Based on our results, microstructural changes of pedunculopontine nucleus and connected subcortical structures are closely related with FOG in PD patients.

1. Introduction

Freezing of gait (FOG) is a gait disorder described as a brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk [1,2]. FOG is a common but disabling symptom in Parkinson's disease (PD) patients, because of its relationship to falling, fracture and impact on quality of life [2]. Furthermore, unlike other parkinsonian symptoms, FOG is hard to manage with medication or surgical treatment [3].

Despite its clinical impact, the pathogenesis of FOG is not yet clearly understood. Two neural networks have recently been suggested for the control of gait based on animal electrophysiology and human brain mapping data [4]. One is a direct pathway from the motor cortex to the spinal cord, and the other is an indirect pathway from the frontal cortex, via the basal ganglia, to brainstem locomotor centers. In PD patients, the direct pathway cannot compensate for all the deficiencies in the indirect pathways, and FOG may develop.

The pedunculopontine nucleus (PPN) is one of the main brainstem locomotor centers and is associated with the regulation of gait and posture [5,6]. Previous studies, using kinetic analysis of gait, reported abnormal gait coordination in PD patients with FOG [7,8] and using imaging analysis, demonstrated that FOG is related with abnormalities in PPN area [9,10]. Furthermore, electrophysiologic abnormalities in PPN area were also reported in PD patients with FOG [6,11].

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique sensitive to the orientation of mobility in intravoxel water molecules [12]. Fractional anisotropy (FA) and mean

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diffusivity (MD) are two of most commonly used DTI measures [13]. FA value is related to isotropic water diffusion when water molecules move in all directions or only in a restricted direction, and MD represents a parameter of average molecular motion. Altered FA and MD values can be applied to microstructural changes of neural tissue in PD [14].

In this study, we compared FA and MD values in the PPN and connected structures, such as basal ganglia, thalamus, cerebellum and frontal cortex, between PD patients with and without FOG.

2. Methods

2.1. Subjects and clinical assessments

This study was approved by the Institutional Review Board of Samsung Medical Center and all subjects provided written informed consent. PD patients and age/sex matched controls were recruited through the movement disorders clinic at Samsung Medical Center, Seoul, Korea. All PD patients were diagnosed with PD using the United Kingdom PD Society Brain Bank criteria, and divided into PD patients with FOG (p-FOG) and without FOG (n-FOG). FOG was evaluated using the FOG questionnaire (FOGQ) [15], and we defined the p-FOG group patients as PD patients with a FOGQ item 3 score $\geq$1 [15], and FOG observed during 10 m walking, turning and going through a narrow doorway [16].

We examined subjects in the 'on' stage using the Unified Parkinson Disease Rating Scale (UPDRS) part III, Hoehn and Yahr (H&Y) stage to evaluate their parkinsonism. PD patients were divided into 3 groups (tremor dominant, akinetic-rigid and mixed) based on their main motor symptoms [17]. We used the Korean mini-mental status exam (MMSE-K) establish cognitive status. White matter changes were assessed using age-related the white matter change (ARWMC) rating scale on axial T2 images of conventional magnetic resonance imaging (MRI) [18]. To identify changes in DTI data due to PD itself, we compared DTI data of PD patients with age, sex, MMSE-K and ARWMC scores matched normal controls, and to focus on the changes related with FOG, age, sex, disease duration, MMSE-K, UPDRS part III score, H&Y stage and ARWMC scores were matched between the p-FOG and n-FOG groups.

We excluded subjects with cognitive impairment (MMSE-K $\leq$24) or other medical conditions that could affect gait including: depression, stroke, ataxia, apraxia, atypical parkinsonism or joint disease (knee, hip or spine).

2.2. Scan acquisition and imaging data processing

MRI was performed on a 3.0 T Intera Achieva scanner (Philips Healthcare, Best, The Netherlands). Sets of axial diffusion-weighted single-shot echo-planar were collected with the following parameters: 128 $\times$ 128 acquisition matrix; 1.72 $\times$ 1.72 $\times$ 2 mm^3 voxels; reconstructed to 1.72 $\times$ 1.72 $\times$ 2 mm^3; 70 axial slices; 22 $\times$ 22 cm^2 field of view; TE 60 ms, TR 7696 ms; flip angle 90°; slice gap 0 mm; b-factor of 600 s/mm^2. With the baseline image without weighting [0,0,0], diffusion-weighted images were acquired from 45 different directions. All axial sections were acquired parallel to the anterior commissure-posterior commissure line.

DTI-data were processed using FMRIB's Software Library (FSL v4.1.2) software (http://www.fmrib.ox.ac.uk/fsl). We corrected for motion artifacts and eddy current distortions by normalizing each diffusion-weighted volume to the non-diffusion-weighted volume (b0) using affine registration in the FMRIB's Linear Image Registration Tool (FLIRT). Before fitting the tensor, brain masks of b0 image were created using the brain-extraction tool (BET v2.1) with 0.2 of fractional threshold to remove background noise [19]. After correcting eddy current distortion and generating brain mask, we calculated the diffusion tensor with the DTIFIT program in FSL for whole brain volumes and maps of FA and MD were determined for every voxel according to standard methods.

2.3. DTI analyses

We used whole-brain voxel-based analysis (VBA), a fully automated whole-brain analysis that uses voxel-wise statistics on diffusion metrics [20] and regions of interest (ROI) analysis due to the small size of PPN to investigate the neural damage of PPN [21–23].

For whole-brain VBA, customized template image creation and analysis procedure were carried out using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/) [20]. For the customized template creation, the B0 DTI volumes from the whole subjects were spatially normalized to the standard T2-MRI template established by Jones et al. [24]. The spatially normalized B0 images were averaged to provide the group template in stereotactic space. The parameters resulting from this spatial normalization step were then applied to the FA and MD map of each subject. In this manner we obtained the normalized FA and MD maps of all subjects; their voxel size was 2 $\times$ 2 $\times$ 2 mm^3 in common Talairach space. The registered FA and MD maps were smoothed with an isotropic Gaussian kernel (8 mm) full width at half maximum, and the smoothed images were compared between the PD patients and normal controls, and between p-FOG and n-FOG group.

We investigated MD and FA values of PPN using ROI analysis. ROI was traced by 3 investigators (J Y, TO S, JW C) who were blinded to the subjects' clinical data. PPN was identified as a single seed voxel between superior cerebellar peduncle and medial lemniscus on red-green-blue image using FSL v4.1.2 software [21–23].

2.4. Statistical analyses

All demographic and clinical data are presented as mean $\pm$ standard deviation. Differences between the p-FOG and n-FOG group were evaluated using unpaired Student's *t*-test or Mann–Whitney *U* test for continuous and ordinal variables, while Pearson's chi-square test or Fisher's exact test were used to determine categorical variables. Inter-observer repeatability for ROI positioning of PPN was assessed by interclass coherence coefficient. To identify the relationship between microstructural changes of PPN and FOG severity or general cognition, partial Spearman correlation analyses were carried out between FA or MD values, and FOGQ or K-MMSE score, with other clinical data, including UPDRS part 3 score and age, as controlled variables. We rejected the null hypotheses of no difference if *p*-values were <0.05. All statistical analyses were conducted using commercially available software (PASW for Windows, version 18.0; SPSS, Chicago, IL, USA).

In whole-brain VBA, DTI images between the p-FOG and n-FOG group were compared using voxel-wise two-sample *t*-test statistics by SPM5. The SPM (*t*) maps from FA and MD images were transformed to the unit of normal distribution, and the *P* values were calculated. The threshold of the *p*-value was 0.05 (False-discovery rate corrected). Only clusters of more than 30 contiguous voxels were considered in the analysis. This combination of a level and cluster size was chosen to provide protection against type I error.

3. Results

3.1. Demographic and clinical data

We enrolled thirty three normal controls and 42 PD patients. The PD patients were divided into two groups; 19 in p-FOG group and 23 in n-FOG group. There was no significant difference in demographic and clinical data between PD patients and normal controls, or between the p-FOG and n-FOG groups (Table 1). Even though the mean age and disease duration of the p-FOG group were higher than those of n-FOG group, there was no significant difference. The total FOGQ score in the p-FOG group was 13.1 $\pm$ 4.65 and the mean score of FOGQ item 3 was 3.3 $\pm$ 0.78. FOG was more prominent when the patients were initiating gait than when turning (mean score of FOGQ item 5 was 2.1 $\pm$ 1.20 and 6 was 1.2 $\pm$ 1.03, $p = 0.035$).

3.2. DTI analyses between PD patients and normal controls

When we compared the DTI data between the PD patients and normal controls, FA values in left thalamus, bilateral orbitofrontal area and bilateral substantia nigra were significantly lower in PD patients than normal controls (Fig. 1). PD patients showed higher MD values in bilateral inferior temporal cortex, orbitofrontal cortex, insula and left frontal area in whole-brain analysis than normal controls.

3.3. DTI analyses between p-FOG and n-FOG group

In whole-brain VBA, the p-FOG group demonstrated a higher MD value in basal ganglia, thalamus and cerebellum than n-FOG group, while there was no difference in cerebral cortex (Fig. 2). For FA values, there were no differences between two groups. Using regions of interest analysis, p-FOG group showed reduced FA values and higher MD values in bilateral PPN than n-FOG group (Table 2).

In correlation analysis, there was no correlated anatomic region with either the sum score or any item score of FOGQ using VBA. In partial correlation analysis with age, K-MMSE, UPDRS part III scores as controlled variables, FA value in right PPN was moderately correlated with FOG severity (sum of FOGQ score) and FOG during turning (FOGQ item 6) (Table 3). Bilateral FA values were also moderately correlated with general status of gait (FOGQ item 1 or 2). MD value in right PPN was correlated with general status of gait