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ORIGINAL ARTICLE

# Structural brain connectivity in patients with restless legs syndrome: a diffusion tensor imaging study

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## Abstract

**Study Objectives:** To evaluate alterations of global and local structural brain connectivity in patients with restless legs syndrome (RLS).

**Methods:** Patients with primary RLS and healthy controls were recruited at a sleep center where they underwent diffusion tensor imaging (DTI) of the brain. We calculated the network measures of global and local structural brain connectivity based on the DTI in both groups using DSI studio program and a graph theory.

**Results:** A total of 69 patients with primary RLS and 51 healthy controls were included in the study. We found a significant difference in the global structural connectivity between the groups. The transitivity in the patients with RLS was lower than that in healthy controls (0.031 vs. 0.033, $p = 0.035$). Additionally, there were significant differences in the local structural connectivity between the groups. The characteristic path length ($r = 0.283$, $p = 0.018$), radius of graph ($r = 0.260$, $p = 0.030$), and diameter of graph ($r = 0.280$, $p = 0.019$) were all positively correlated with RLS severity, whereas the mean clustering coefficient ($r = -0.327$, $p = 0.006$), global efficiency ($r = -0.272$, $p = 0.023$), small-worldness index ($r = -0.325$, $p = 0.006$), and transitivity ($r = -0.351$, $p = 0.003$) were negatively correlated with RLS severity.

**Conclusion:** We identified changes in the global structural connectivity of patients with RLS using graph theory based on DTI, which showed decreased segregation in the brain network compared to healthy controls. These changes are well correlated with RLS severity. We also found changes in local structural connectivity, especially in regions involved in sensorimotor function, which suggests that these areas play a pivotal role in RLS. These findings contribute to a better understanding of the pathophysiology of RLS symptoms.

### Statement of Significance

We explored the changes in the structural connectivity of patients with primary RLS compared to healthy controls using DTI and graph theory. Decreased segregation in the brain network of the patients with RLS was observed. In addition, these changes are well correlated with RLS severity. We also found changes in local structural connectivity, especially in regions involved in sensorimotor function, which suggests that these areas play a pivotal role in RLS. These findings contribute to a better understanding of the pathophysiology of RLS symptoms.

**Key words:** restless legs syndrome; diffusion tensor imaging; brain

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## Introduction

Restless legs syndrome (RLS) is a common sensorimotor disorder, with a prevalence of 6%–12% [1]. It is characterized by intensely unpleasant sensations and an almost implacable desire to move the legs in the evening or at night [2, 3]. Symptomatic relief can be temporary and may be partially achieved through leg movement or walking [2, 3].

The pathophysiology of the RLS is still poorly understood. Studies examining the in vivo morphological correlations between RLS and pathophysiology of the central nervous system have been conducted using brain magnetic resonance imaging (MRI) [4]. A voxel-based morphometry study using T1-weighted MRI to detect region-specific differences in gray matter between patients with RLS and controls reported a bilateral increase in gray matter in the thalamus, particularly in the pulvinar areas of patients with RLS [5]. The authors hypothesized that these structural changes in the thalamus might be involved in the pathogenesis of RLS or a result of chronically increased afferent input of behaviorally relevant information [5]. A whole-brain tract-based spatial statistics study based on diffusion tensor imaging (DTI) reported that compared to healthy controls, patients with RSL had decreased axial diffusivity and fractional anisotropy (FA) in the thalamic, corona radiates, and corticospinal tract, which are areas associated with sensory or motor function [6, 7], and another study reported that patients with RLS had decreased FA in the genu of the corpus callosum and frontal white matter adjacent to the inferior frontal gyrus [8]. These findings lend support to the hypothesis of defective integration within the sensorimotor network in RLS [6, 7]. In addition, significantly altered microstructure of the midbrain in patients with RLS has been demonstrated by a DTI study [9].

With the advent of network analysis, our understanding of the brain and various neurological disorders has significantly improved. Subsequently, recent interest has centered on studies of brain network or connectivity changes in patients with RLS. A few studies have examined functional connectivity changes in patients with RLS using resting-state functional MRI (rs-fMRI). They reported significant changes in a variety of brain networks, including the thalamic [10], salience [11], default mode [12], and small-world networks [13], in patients with RLS when compared to healthy controls. Additionally, network changes associated with RLS treatments have been discovered, including repetitive transcranial magnetic stimulation [14], transcutaneous spinal cord direct current stimulation [15], and dopaminergic drugs [16]. These findings imply that the underlying pathogenesis of RLS may involve changes in the brain's functional connectivity and that RLS may be a network disorder.

Two brain regions are structurally connected if they are physically connected by a fiber tract. Typically, this is determined in vivo using DTI. Structural connectivity can be measured using several quantitative connectivity values, which are computed from a set of streamlines corresponding to a particular pathway. The objective of measuring structural connectivity is to approximate the true underlying fiber density or axon count. However, no studies have investigated changes in the structural connectivity of patients with RLS.

In this study, we aimed to evaluate the changes in the structural connectivity of patients with primary RLS compared to healthy controls using DTI and graph theory. We hypothesized that there were significant changes in global and local structural connectivity, and that these changes were correlated with RLS severity in patients with primary RLS.

## Methods

### *Participants: patients with RLS and healthy controls*

Patients and healthy controls were recruited from a specialized sleep center in a tertiary hospital. The study was approved by the institutional review board, and all participants provided written informed consent. We enrolled patients with RLS according to the following criteria: (1) primary RLS diagnosed by a certified Korean neurologist, an expert in RLS, during face-to-face interviews according to the criteria defined by the International RLS Study Group (IRLSSG) [17], (2) no structural lesions on their brain MRI, and (3) no other medical or neurological diseases affecting DTI, except RLS. We excluded the patients with secondary RLS due to iron deficiency anemia, pregnancy, chronic kidney disease, or peripheral neuropathy. In addition, we enrolled healthy subjects as a control group, whose age and sex were similar to the patients with RLS. The healthy controls all answered "no" on the initial questions of the RLS diagnosis questionnaire [17]. They had normal brain MRIs and no medical or neurological diseases.

For the patients with RLS, the severity of RLS symptoms was assessed using the International RLS scale [18]. Additionally, the following scales were assessed with sleep questionnaires: (1) the Restless Legs Syndrome Quality of Life Questionnaire [19], (2) the Insomnia Severity Index [20], (3) the Pittsburgh Sleep Quality Index [21], and (4) the Hospital Anxiety and Depression Scale [22].

### *DTI acquisition*

Both patients with RLS and healthy controls underwent DTI using a 3.0T MRI scanner equipped with a 32-channel head coil (AchievaTx, Philips Healthcare, Best, The Netherlands). The specific DTI parameters were as follows: 32 different diffusion directions, *b*-values of 0 and 1000 s/mm$^2$ (b0 images were acquired once), TR/TE = 8620/85 ms, FA = 90°, slice thickness = 2.25 mm, acquisition matrix = 120 × 120, field of view = 240 × 240 mm$^2$, parallel imaging factor (SENSE) of 2. The phase direction was set to anterior-posterior and the fat shift direction to posterior.

### *Structural connectivity analysis*

We calculated the network measures of global and local structural connectivity in patients with RLS and healthy controls using DSI studio program (https://dsi-studio.labsolver.org) (Figure 1). Initially, we opened source DTI images and created a mask with the following steps: thresholding, smoothing, and defragment. We also conducted preprocessing with FSL's top-up and eddy to handle susceptibility artifacts and eddy current distortion. Then, we generated one fiber orientation per voxel and associated anisotropy and diffusivity measure with generalized q-sampling imaging reconstruction to T1-weighted (T1W) space. This approach handles the individual difference in brain parcellation, but fiber tracking is done in the native space. The diffusion data are reconstructed to subject's T1W space so that all following analysis is in the T1W coordinates. We performed fiber tacking by seeding the whole-brain region. A deterministic fiber tracking algorithm was used. Finally, we performed spatial normalization to ensure that the built-in parcellation atlas with AAL3 [23] was registered with the subject data. Network

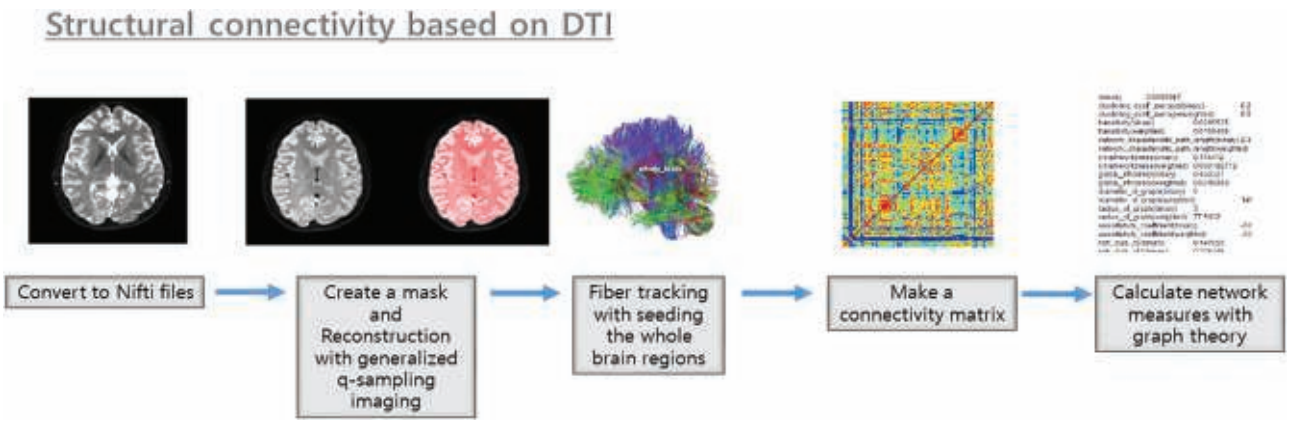


**Figure 1.** The process for structural connectivity analysis in this study.

**Table 1.** Clinical characteristics of patients with restless legs syndrome and healthy controls

| | Patients with RLS (*N* = 69) | Healthy controls (*N* = 51) | *p* |
|---|---|---|---|
| Age, years | 57.0 ± 6.7 | 55.9 ± 8.1 | 0.443 |
| Male, *n* (%) | 20 (28.9) | 13 (25.4) | 0.672 |
| Age of onset, years | 47 (41.7–54.0) | | |
| Symptom duration, months | 120 (39–162) | | |
| RLS severity | 27.1 ± 6.4 | | |
| Disease-specific quality of life | 8.7 ± 3.3 | | |
| PSQI | 12 (9.0–14.2) | 4 (3–5) | <0.001 |
| ISI | 16 (11–23) | 3 (1–4) | <0.001 |
| HAS | 7 (4–9) | 4 (2.2–5) | <0.001 |
| HDS | 8 (5–11) | 5 (4–7) | 0.007 |

RLS, restless legs syndrome; PSQI, Pittsburgh Sleep Quality Index; ISI, Insomnia Severity Index; HAS, Hospital Anxiety Scale; HDS, Hospital Depression Scale.

measures were calculated with graph theory. The connectivity matrix was normalized to choose the weighed measures, such that the maximum value of the matrix was one. The global structural connectivity was assessed with measures, such as assortative coefficient, mean clustering coefficient, diameter of graph, global efficiency, characteristic path length, radius of graph, small-worldness index, and transitivity, and the local structural connectivity was investigated with the measure including betweenness centrality [24–26].

### *Correlation analysis*

We conducted a correlation analysis between the measures of the global structural connectivity, including the assortative coefficient, mean clustering coefficient, diameter of graph, global efficiency, characteristic path length, radius of graph, small-worldness index, and transitivity, and the betweenness centrality in regions showing significant differences between the groups, and the RLS severity in the patients with RLS.

### *Statistical analysis*

Categorical variables were presented as frequency with percentage, and continuous variables were presented as mean value with standard deviation (*SD*) or median with interquartile range depending on normal distribution. The categorical variables were analyzed using the chi-square test, and continuous variables were tested with the independent samples *t*-test or the Mann-Whitney test according to normal distribution. The correlation analysis was conducted with Pearson's test. Multiple corrections with false discovery rate (Benjamini-Hochberg procedure) were applied in the analysis of the global and local structural connectivity and in the correlation analysis. Then, the adjusted *p*-value was represented. The *p*-values below 0.05 were considered statistically significant. Statistical analyses were performed using MedCalc Statistical Software version 20.014 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). We also used R-4.1.3 program for applying multiple corrections.

## Results

### *Clinical characteristics*

A total of 69 patients with primary RLS and 51 healthy controls were included in the study. Table 1 shows the clinical characteristics of all the participants. There were no significant differences in the age and sex (57.0 vs. 55.9 years, $p = 0.443$; Male, 20/69 (28%) vs. 13/51 (25%), $p = 0.672$) between the two groups. More than two-thirds of the patients with RLS were women.

### *Global structural connectivity*

Table 2 shows the differences in the global structural connectivity between the patients with RLS and the healthy controls. There was a significant difference in the measure

**Table 2.** The differences in global structural connectivity between patients with restless legs syndrome and healthy controls

| | Patients with RLS (*N* = 69) | | Healthy controls (*N* = 51) | | | | |
|---|---|---|---|---|---|---|---|
| Network measures | Mean | *SD* | Mean | *SD* | Difference | *p* | Adjusted *p* |
| Assortative coefficient | 0.026 | 0.028 | 0.029 | 0.023 | 0.003 | 0.569 | 0.602 |
| Mean clustering coefficient | 0.034 | 0.004 | 0.035 | 0.005 | 0.000 | 0.488 | 0.602 |
| Diameter of graph | 111.485 | 48.621 | 122.094 | 58.614 | 10.609 | 0.421 | 0.602 |
| Global efficiency | 0.079 | 0.009 | 0.078 | 0.010 | –0.001 | 0.602 | 0.602 |
| Characteristic path length | 18.629 | 2.719 | 19.200 | 3.123 | 0.571 | 0.349 | 0.602 |
| Radius of graph | 68.125 | 37.978 | 72.977 | 39.041 | 4.852 | 0.462 | 0.602 |
| Small-worldness index | 0.002 | 0.000 | 0.002 | 0.000 | 0.000 | 0.517 | 0.602 |
| Transitivity | 0.031 | 0.004 | 0.033 | 0.004 | 0.002 | 0.006 | 0.048 |

RLS, restless legs syndrome.

of transitivity. The transitivity in the patients with RLS was lower than that in healthy controls (0.031 vs. 0.033, $p = 0.048$). However, the other measures of global structural connectivity, including assortative coefficient (0.026 vs. 0.029, $p = 0.602$), mean clustering coefficient (0.034 vs. 0.035, $p = 0.602$), diameter of graph (111.485 vs. 122.094, $p = 0.602$), global efficiency (0.079 vs. 0.078, $p = 0.602$), characteristic path length (18.629 vs. 19.200, $p = 0.602$), radius of graph (68.125 vs. 72.977, $p = 0.602$), and small-worldness index (0.002 vs. 0.002, $p = 0.602$) were not different between patients with RLS and healthy controls.

### *Local structural connectivity*

There were significant differences in the local structural connectivity between the patients with RLS and healthy controls (Suppl. 1). Figure 2 visualizes the regions with significant differences in the local structural connectivity between the two groups. The betweenness centrality in the right middle frontal gyrus (193.649 vs. 263.815, $p = 0.017$), left media part of superior frontal gyrus (181.656 vs. 238.487, $p = 0.028$), right hippocampus (354.467 vs. 423.752, $p = 0.038$), left medial orbital frontal gyrus (6.010 vs. 10.859, $p = 0.033$), right postcentral gyrus (240.260 vs. 301.870, $p = 0.011$), left pulvinar anterior thalamic nucleus (39.000 vs. 58.694, $p = 0.022$), and right pulvinar anterior thalamic nucleus (63.461 vs. 85.795, $p = 0.040$) were decreased in patients with RLS compared to healthy controls. Whereas the betweenness centrality in the left nucleus accumbens (62.346 vs. 38.269, $p = 0.019$), right supplementary motor area (218.177 vs. 174.466, $p = 0.046$), and lobule IV, V of vermis (135.114 vs. 102.091, $p = 0.008$) were increased in the patients with RLS compared to healthy controls.

### *Correlations between measures of global structural connectivity and RLS severity*

We found significant correlations between the measures of global structural connectivity and RLS severity (Figure 3). The characteristic path length ($r = 0.283$, $p = 0.032$), radius of graph ($r = 0.260$, $p = 0.035$), and diameter of graph ($r = 0.280$, $p = 0.032$) were all positively correlated with RLS severity, whereas mean clustering coefficient ($r = –0.327$, $p = 0.017$), global efficiency ($r = –0.272$, $p = 0.032$), small-worldness index ($r = –0.325$, $p = 0.017$), and transitivity ($r = –0.351$, $p = 0.010$) were negatively correlated with RLS severity. However, there was no significant correlation between the assortative coefficient and RLS severity ($r = –0.063$, $p = 0.605$).

Furthermore, there were no significant correlations between the betweenness centrality of the right middle frontal gyrus ($r = –0.125$, $p = 0.886$), left media part of superior frontal gyrus ($r = –0.096$, $p = 0.886$), right hippocampus ($r = –0.095$, $p = 0.886$), left medial orbital frontal gyrus ($r = 0.017$, $p = 0.886$), right postcentral gyrus ($r = 0.050$, $p = 0.653$), left pulvinar anterior thalamic nucleus ($r = –0.065$, $p = 0.886$), right pulvinar anterior thalamic nucleus ($r = 0.019$, $p = 0.886$), left nucleus accumbens ($r = 0.053$, $p = 0.886$), right supplementary motor area ($r = –0.071$, $p = 0.886$), and lobule IV, V of vermis ($r = –0.053$, $p = 0.886$), and RLS severity.

## Discussion

This was the first study to compare the changes in the structural connectivity of patients with primary RLS with healthy controls using DTI and graph theory. The main findings of this study were (1) evidence of significant changes in the global structural connectivity of patients with RLS compared to healthy controls, (2) the presence of several regions with significant differences in local structural connectivity between the groups, and (3) significant correlations between the measures of global structural connectivity and RLS severity.

We investigated the difference in the global structural connectivity between the patients with RLS and healthy controls using graph theory, and the transitivity in the patients with RLS was significantly decreased compared to that of healthy controls. A graph's transitivity is determined by the proportion of triangles in the graph to the total number of connected triples of nodes [25, 27]. Transitivity is the probability of adjacent nodes being connected in a network, indicating the presence of tightly connected communities, and it is a global measure of overall efficiency of local processing, termed as a segregation, in the brain [25, 27]. Thus, our results suggest the presence of decreased global structural connectivity, especially segregation, in patients with RLS compared to healthy controls. This finding is in line with a previous functional connectivity study of patients with RLS [28]. Choi et al. investigated the alterations in the brain topology of patients with RLS compared to healthy controls using electroencephalography and graph theory, and they observed disruption of the small-world network [28]. The human brain possesses the characteristics of a small-world network, which facilitates efficient information segregation and integration

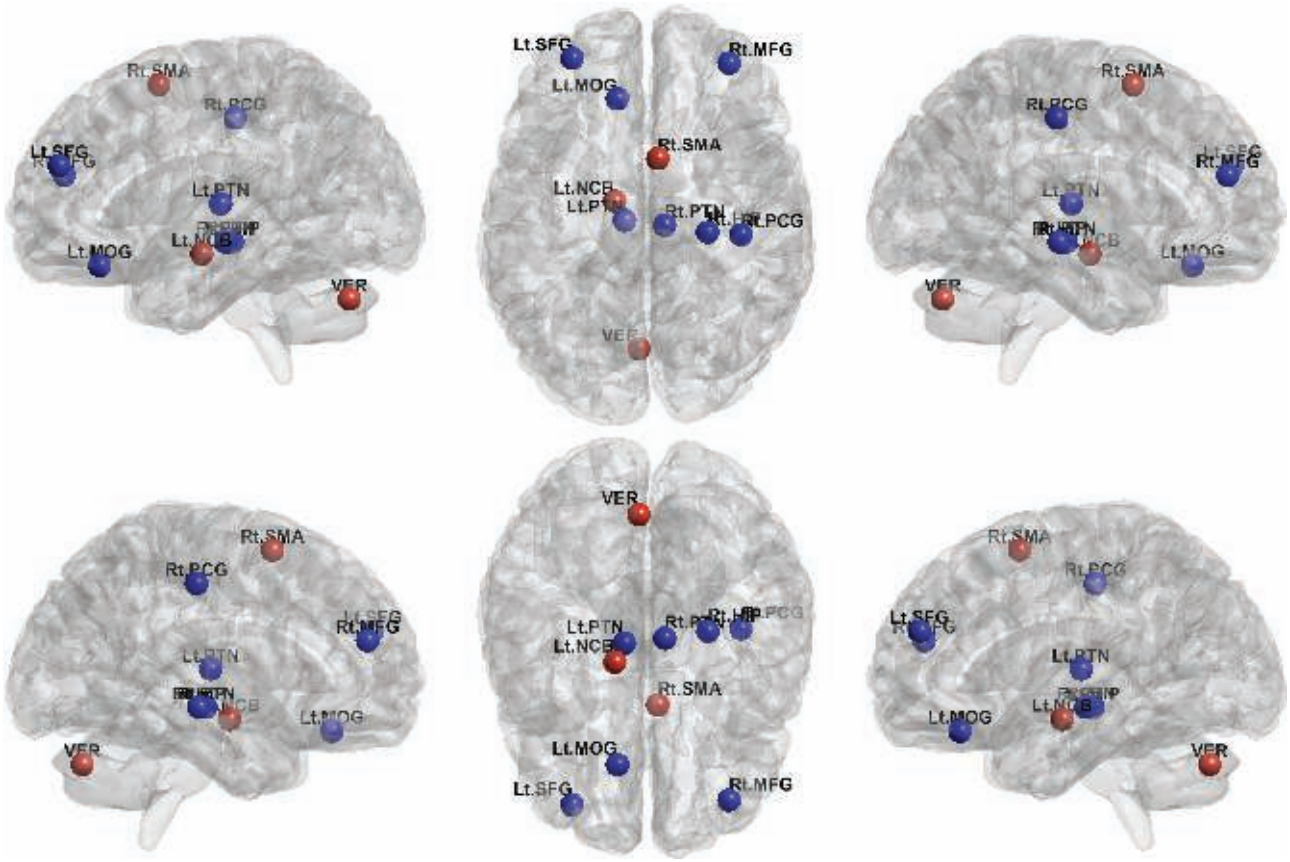


**Figure 2.** The regions with significant differences in the local structural connectivity of patients with restless legs syndrome (RLS) and healthy controls. The blue spheres represent the nodes with decreased betweenness centrality, whereas the red spheres indicate the nodes with increased betweenness centrality in patients with RLS compared to healthy controls. Betweenness centrality in the right middle frontal gyrus, left media part of superior frontal gyrus, right hippocampus, left medial orbital frontal gyrus, right postcentral gyrus, left pulvinar anterior thalamic nucleus, and right pulvinar anterior thalamic nucleus was decreased, whereas the betweenness centrality in the left nucleus accumbens, right supplementary motor area, and lobule IV, V of vermis was increased in patients with RLS compared to healthy controls. Rt. MFG, right middle frontal gyrus, Lt. SFG, left media part of superior frontal gyrus, Rt. HIP, right hippocampus, Lt. MOG, left medial orbital frontal gyrus, Rt. PCG, right postcentral gyrus, Lt. PTN, left pulvinar anterior thalamic nucleus, Rt. PTH, right pulvinar anterior thalamic nucleus, Lt. NCB, left nucleus accumbens, Rt. SMA, right supplementary motor area, VER, lobule IV, V of vermis.

at low cost, and it can be considered an optimal structure for interregional information transfer [29]. This disruption of the small-world network in patients with RLS was caused by decreased local clustering although the path length remained unchanged [28]. Segregation can be quantified using the measure of transitivity (global measure, one value for whole brain per participant), or the clustering coefficient and local efficiency (both local measures, one value per node/region per participant) [30]. Thus, these studies suggest a decrease in the structural and functional segregation in patients with RLS.

Interestingly, we found significant correlations between the measures of global structural connectivity and RLS severity. The characteristic path length, radius of graph, and diameter of graph were all positively correlated with RLS severity, whereas mean clustering coefficient, global efficiency, small-worldness index, and transitivity were negatively correlated with RLS severity. The characteristic path length is defined as the average of all path lengths, which is the average distance between any two nodes [25, 27, 29–31]. The radius of a graph is equal to the minimum eccentricity, the maximum distance between any two nodes, of all nodes, while the diameter of a graph is equal to the maximum eccentricity of all nodes [25, 27, 29–31]. Thus, as the values of the characteristic path length, radius of graph, and diameter of graph increase, the integration and segregation of the network decreases, which results in an eventual decrease in the global structural connectivity. However, the values of the mean clustering coefficient, global efficiency, small-worldness index, and transitivity are applied in the opposite way. The mean clustering coefficient is the average of all clustering coefficients, which is calculated as the ratio of the number of triangles surrounding a node to the maximum number of triangles that could possibly form around that node [25, 27, 29–31]. Global efficiency is the average of all nodes' inverse shortest paths, and the small-worldness index is a characteristic path length that is similar to that of a random graph with the same degree distribution but is significantly more clustered [25, 27, 29–31]. The more severe the RLS symptoms, the more characteristic path length, radius of graph, and diameter of graph increase means that the global brain efficiency decreases, whereas the more severe the RLS symptoms, the less mean clustering coefficient, global efficiency, small-worldness index, and transitivity also means that the global brain efficiency decreases. All these results suggest that global brain efficiency decreases as RLS severity increases.

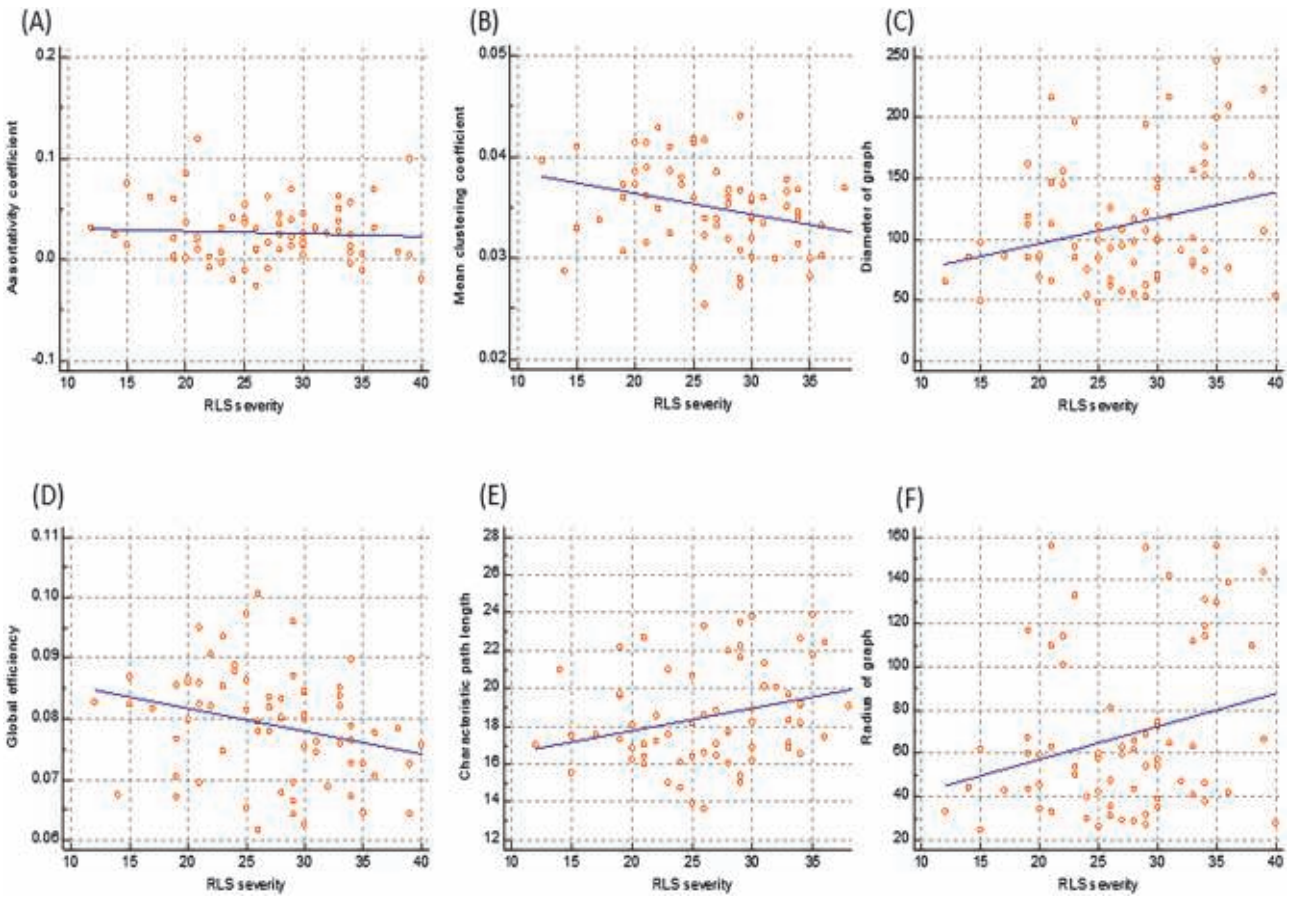


**Figure 3.** Correlations between the measures of global structural connectivity and restless legs syndrome (RLS) severity. There was no significant correlation between the assortative coefficient and RLS severity in patients with RLS (A). However, the mean clustering coefficient (B), diameter of graph (C), global efficiency (D), characteristic path length (E), radius of graph (F), small-worldness index (G), and transitivity (H) were significantly correlated with RLS severity.

Our results also identified changes in the local structural connectivity of patients with RLS compared to healthy controls. Most of the changes were in areas of the brain involved in sensorimotor function, including the middle frontal gyrus, superior frontal gyrus, orbital frontal gyrus, postcentral gyrus, pulvinar anterior thalamic nucleus, and supplementary motor area. Our results on altered sensorimotor network, which may be an underlying cause for the pathophysiology of RLS, are consistent with the results of the previous DTI and rs-fMRI studies. Brain regions associated with sensorimotor function in patients with RLS were reported to differ from that of healthy controls in previous DTI studies. A DTI study of 12 patients with primary RLS reported areas of altered FA in the internal capsule, which includes the corticospinal tract to lower limbs, suggesting changes in sensorimotor pathways associated with RLS [32]. Another study statistically compared the data of 30 healthy controls and 30 patients with RLS using the computation of regional FA [33]. They observed multiple subcortical areas with significantly reduced FA in close proximity to the primary and associated sensorimotor cortex and thalamus in the patient group [33]. Evidence for morphologic changes in the primary somatosensory system in patients with RLS was already demonstrated in a previous study. They demonstrated that patients with RLS had a morphologic change in their brain's somatosensory system when compared to controls, and found that the average cortical thickness in the bilateral postcentral gyrus decreased by 7.5% in patients with RLS compared to healthy controls [34]. These changes in the sensorimotor areas of patients with RLS were also reported in functional connectivity studies. Ku et al. explored the intrinsic changes in the thalamocortical circuit of patients with RLS using rs-fMRI, and they found altered thalamic connectivity in several regions including precentral gyrus and medical frontal gyrus [10]. Another graph theoretical analysis study using rs-fMRI reported a significantly high clustering coefficient and local efficiency in motor and frontal regions, whereas a low clustering coefficient was found in the central sulcus [13]. When patients on dopaminergic medication were compared to untreated patients and controls, connectivity between the thalamus and frontal regions was significantly increased [13]. A recent meta-analysis of rs-fMRI study also demonstrated an altered functional connectivity within the dopaminergic pathways and thalamus. The authors assumed that sensorimotor dysfunction in RLS was reflected by decreased functional connectivity within the dopaminergic pathways, whereas increased functional connectivity in the thalamus could be regarded as an adaptation process to somatosensory dysfunction in RLS [35].

Although we successfully demonstrated changes in the global and local structural connectivity of patients with RLS, this study has several limitations. First, although we enrolled a large sample of patients with primary RLS and healthy

controls, this study was conducted in a single specialized sleep center in a tertiary hospital. Thus, the generalizability of our results is limited. Second, some of the enrolled patients with RLS were already taking medications for RLS. Although there are no studies on structural connectivity changes from RLS treatment, it is considered to have an effect. Functional connectivity studies have found network changes related to RLS treatment, including repetitive transcranial magnetic stimulation, transcutaneous spinal cord direct current stimulation, and dopaminergic drugs [14–16]. Further studies with newly diagnosed patients with RLS in multi-centers are needed to confirm our findings. Lastly, we applied the false discovery rate for multiple corrections using the Benjamini-Hochberg procedure. The false discovery rate is a way of conceptualizing the rate of type I errors when performing multiple comparisons under the null hypothesis. Its procedures have a less stringent control over Type I errors than familywise error rate procedures (such as the Bonferroni correction), which control the probability of at least one Type I error. Thus, false discovery rate-controlling procedures gain in strength at the expense of increased Type I errors.

## Conclusion

We found changes in the global structural connectivity of patients with primary RLS using graph theory, which shows decreased segregation in the brain network compared to healthy controls. In addition, these changes are well correlated with RLS severity. We also identify the changes in local structural connectivity, especially regions involved in sensorimotor function, which suggests that these areas play a pivotal role in RLS. These findings contribute to a better understanding of the pathophysiology of RLS symptoms.

## Supplementary Material

Supplementary material is available at *SLEEP* online.

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## Author Contributions

K.M.P. and K.T.K. equally contributed to paper writing and data analysis as first authors. Y.W.C., K.T.K., and D.A.L. participated in recruiting patients and controlling and organizing data. Y.W.C. supervised the paper writing. All the authors participated in the analysis and interpretation of data. All the authors revised the manuscript critically and approved the manuscript in its final form.

## Disclosure Statement

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