ANNALS of Clinical and Translational Neurology
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RESEARCH ARTICLE

# Patterns of gray matter alterations in migraine and restless legs syndrome

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**Funding Information**
This project was supported in part by grants from the Ministry of Science and Technology of Taiwan (MOST 105-2314-B-016-004-, MOST 106-2314-B-016 -007 -MY2, MOST 106-2221-E-010-011, MOST 107-2221-E-010 -010 -MY3, MOST 106-2221-E-010 -011 -); Tri-Service General Hospital, Taiwan (TSGH-C101-159, TSGH-C106-068, TSGH-C107-072).

Received: 12 August 2018; Revised: 29 September 2018; Accepted: 1 October 2018

***Annals of Clinical and Translational Neurology*** **2019; 6(1): 57–67**

doi: 10.1002/acn3.680

## Abstract

**Objectives**: Migraine and restless legs syndrome (RLS) are often comorbid and share elements of pathology; however, their neuroanatomical underpinnings are poorly understood. This study aimed to identify patterns of gray matter volume (GMV) alteration specific to and common among patients with RLS, migraine, and comorbid migraine and RLS. **Methods**: High-resolution T1-weighted images were acquired from 116 subjects: 27 RLS patients, 22 migraine patients, 22 patients with comorbid migraine and RLS, and 45 healthy controls. Direct group comparisons and conjunction analysis were first used to localize the distinct and shared neural signatures of migraine and RLS. We also investigated whether the shared neural signature could be replicated in an additional comorbid migraine/RLS group. **Results**: Compared with healthy controls, migraine patients showed GMV changes in the lateral occipital cortex, cerebellum, frontal pole, and middle frontal gyrus (MFG), and RLS patients showed GMV changes in the thalamus, middle temporal gyrus, anterior cingulate cortex, insular cortex, and MFG. In migraine, compared with RLS, GMV differences were found in the precuneus, lateral occipital and occipital fusiform cortex, superior frontal and precentral gyri, and cerebellum. Conjunction analyses for these disorders showed altered GMV in the MFG, also found in patients with comorbid migraine and RLS. The GMV of the MFG also correlated with sleep quality in patients with comorbid migraine and RLS. **Interpretation**: Migraine and RLS are characterized by shared and distinctive neuroanatomical characteristics, with a specific role of the MFG. These findings may be related to shared pathophysiology of these two distinct disorders.

## Introduction

Migraine, a primary headache disorder affecting 10–20% of the general population, is characterized by recurrent attacks of moderate to severe pulsating headache lasting from 4 to 72 h. It is female-predominant, with a female-to-male ratio of 2–3:1. Migraine attacks are often associated with photophobia, phonophobia, or nausea and vomiting, and are aggravated by physical activity.[1] Numerous other factors, including sleep, cardiovascular state, and mood disorders are strongly associated with migraine.[2,3] One of the most common comorbidities is

restless legs syndrome (RLS).[4,5] RLS is a sensorimotor disorder characterized by a deeply uncomfortable sensation in the legs, especially when at rest or near bedtime, and relieved by voluntary movement. The prevalence of RLS is 5–10% in the general population in western countries, but substantially higher in patients with migraine (8.7–39.0%).[6] Furthermore, higher migraine frequency is associated with a higher prevalence of RLS.[7] Although several pathophysiological mechanisms have been proposed linking migraine with RLS, including dopaminergic imbalances, dysfunction in iron metabolism, and genetic background variations,[8,9] little is known about the neural mechanisms governing the comorbidity of the two conditions.

In migraine, neuroanatomical alterations have been reported for a range of brain regions, including the insula; motor/premotor, prefrontal, cingulate, posterior parietal, orbitofrontal, and somatosensory cortices, and the caudate nucleus.[10–12] The brain structural abnormalities in RLS patients are even more diverse, including increased gray matter volume (GMV) in the pulvinar,[13] ventral hippocampus, and middle orbitofrontal gyrus[14] and decreased GMV in the primary somatosensory cortex and primary motor area.[15] However, other studies yielded no specific GMV changes.[16–19] Although it is apparent that widespread brain areas are associated with both migraine and RLS, their common and distinct roles are still unclear.

Few neuroimaging studies have investigated the shared and distinct neural properties of migraine and RLS.[20] In our recent resting-state functional magnetic resonance imaging (rs-fMRI) study with a design comparing three clinical groups of individuals with migraine patients with and without RLS, and healthy controls, we observed disrupted functional connectivity (FC) in patients with migraine with and without RLS when compared with healthy controls.[20] Both groups exhibited FC changes, most prominently in attentional, nociceptive, control, and sensory-related networks. It was unclear, however, whether these functional changes were related to structural changes. Moreover, no patients with RLS only were examined, leaving the question of comorbidity between migraine and RLS unaddressed.

In this study, therefore, to address the limitations of previous research and clarify the neural mechanisms of these two disorders, we used a T1 voxel-based morphometry (VBM) approach and hierarchical study design to identify the shared and distinct neural signature of migraine, RLS, and both (that is, comorbidity). First, direct group comparisons and conjunction analysis were used to localize the distinct and shared neural signature of migraine and RLS. Second, we investigated whether the shared neural signature that appeared when either disease was present without the other, was also replicated in an additional comorbid migraine/RLS group. Finally, associations between the shared neural signature and clinical variables were also evaluated.

## Materials and Methods

### Participants

The study protocol was approved by the Institutional Review Board of Tri-Service General Hospital (TSGH); all participants provided informed written consent before enrollment. In this study, 116 participants were recruited consecutively from the Headache Clinic, Neurology Department of TSGH: 22 participants diagnosed with migraine (81.8% female; migraine with aura $n = 4$; migraine without aura, $n = 18$), 27 with RLS (74.0% female), 22 with comorbid migraine and RLS (95.4% female; migraine with aura, $n = 5$; migraine without, aura $n = 17$), and 45 healthy controls (73.3% female) with no history of neurological or psychiatric disease.

The diagnosis of migraine was defined according to the third edition of the International Classification of Headache Disorders third edition (ICHD-III).[1] Secondary or other concomitant primary headache disorders were excluded. In addition, we documented the clinical characteristics of participants diagnosed with migraine, including migraine duration, frequency, aura symptoms, family history, and headache intensity. Primary RLS was diagnosed based on the criteria outlined by the International Restless Legs Syndrome Study Group (IRLSSG),[21] after detailed history survey, physical examination, laboratory tests, and electromyography to exclude secondary RLS due to iron deficiency anemia, pregnancy, chronic renal disease, diabetes, peripheral neuropathy, radiculopathy, Parkinson's disease, psychiatric illness, head injury, serious medical conditions, or history of drug or alcohol dependence. No patient was treated with dopaminergic agents, antidepressants, neuroleptics, or hypnotics. Other demographic and clinical data, including gender, age, RLS and migraine duration, and sleep quality (Pittsburgh Sleep Quality Index [PSQI]) were also recorded. RLS severity was assessed using the IRLSSG rating scale.[21] No acute migraine or RLS attacks occurred during the scanning sessions. The healthy control group comprised volunteers without RLS or migraine, matched for age, gender, and handedness. Control participants were recruited through community advertisements and hospital patient pools. Exclusion criteria for control participants included a family history of RLS or migraine, prior diagnosis of a primary or secondary headache disorder, and any chronic pain condition.

 

## MRI data acquisition

All T1-weighted (T1w) structural scans were acquired at the TSGH on the same 3-T Discovery MR750 scanner (General Electric Healthcare, Milwaukee, WI, USA) using an eight-channel head array coil for signal detection. Axial three-dimensional inversion recovery prepared fast spoiled gradient recalled (IR-FSPGR) sequence with the following parameters was used for image acquisition: repetition time/echo time/inversion time = 10.17/4.16/450 msec; flip angle = 12°; number of excitations = 1; field of view = 256 × 256 $mm^2$; matrix size = 256 × 256; 172 slices and 1 mm isotropic voxel size (without interpolation and interslice gap). An experienced neuroradiologist checked the raw anatomical scans visually to ensure that no gross brain abnormalities existed. To minimize variability in scanning position and further improve registration accuracy across study participants, we used a center-of-mass approach to reorient the image origin of each T1w scan automatically.

## Diffeomorphic anatomical registration exponentiated lie algebra VBM

To localize GMV changes among study groups, the VBM analytical framework was used.[22] Native-space reoriented T1w scans were preprocessed with the Computational Anatomy Toolbox (CAT12, version 1266, http://www.neuro.uni-jena.de/cat/) of Statistical Parametric Mapping software (SPM12, version 7219, Wellcome Institute of Neurology, University College London, UK, http://www.fil.ion.ucl.ac.uk/spm/) in Matlab R2016a (The Mathworks, Inc., Natick, MA, USA) with default settings. All T1w scans were corrected for intensity nonuniformities, and then segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) compartments based on an adaptive maximum a posteriori method[23] and partial volume estimation model.[24] The resulting individual GM and WM tissue segments were spatially normalized to create study-specific Montreal Neurological Institute (MNI)-space tissue templates, using the high-dimensional Diffeomorphic Anatomical Registration Exponentiated Lie Algebra (DARTEL) normalization approach.[25] As a high-dimensional warping method, DARTEL estimates the deformation field between native space and MNI space for each individual. To obtain absolute tissue volume, the resulting MNI-space GM tissue segments were further modulated by the linear and nonlinear components of the Jacobian determinant obtained from the DARTEL deformation fields. Finally, the resulting MNI-space-modulated GM images were spatially smoothed by convolving with an isotropic 8-mm full-width-at-half-maximum Gaussian filter. The spatial resolution of the final preprocessed GM segments was 1.5 $mm^3$. After completing the entire VBM processing pipeline and visual inspection for incorrect preprocessing, the "Check Data Quality" module of CAT12 was used to assess GM segment homogeneity and identify possible outliers. No participant was excluded in this step. Each total intracranial volume (TIV) was calculated as the sum of the GM, WM, and CSF volumes in native space and used as a potential confounding factor for adjusting global variation in brain size. We used a general linear model to remove the nuisance effects of age, gender, and TIV across all study participants, and the resulting GM residual maps were used as inputs for the subsequent statistical analyses. The individual unmodulated GM segments were averaged and thresholded at intensity 0.2 to create an explicit mask for exclusion of artifacts on the border between GM and WM (i.e., partial volume effect).

## Statistical analyses

### Analyses of demographic, clinical evaluations, and neuroimaging data

All statistical analyses of demographic data, clinical evaluations, and global tissue volumes were performed with SPSS Version 20 for Windows (SPSS, Chicago, IL, USA). The demographic data, clinical evaluations, and TIV were compared among the study groups by analysis of variance tests and Pearson's chi-squared test, where appropriate, and were reported as the mean ± standard deviation. Moreover, we performed analysis of covariance with age, gender, and TIV as nuisance variables to compare global tissue volume (GM volume, WM volume, and CSF volume). The GLM_Flex toolbox (http://mrtools.mgh.harvard.edu/index.php?title=GLM_Flex) and SPSS software were used for voxel-wise statistical analyses and region of interest (ROI) analyses respectively. For all voxel-wise statistical analyses, clusters with differences in GMV were considered statistically significant at the cluster-level family wise-error (FWE) corrected $P < 0.05$, with a cluster-forming threshold of a voxel-level $P < 0.005$ and 210 voxel extents. We used the command-line tool 3dClustSim (10,000 permutations with explicit GM mask; version AFNI_17.3.01) to determine the statistical criterion. To ensure transparency and reusability of statistical results, we uploaded all the unthresholded statistical maps to the NeuroVault website (https://neurovault.org/collections/3449/).

 

### Analysis of regional GMV differences between patients with migraine, RLS, and healthy controls to identify shared and distinct anatomical signatures

Voxel-wise t-tests were first performed based on the diagnosis and focused on the statistical contrasts of migraine versus healthy controls, and RLS versus healthy controls. Furthermore, we performed conjunction analysis to identify anatomical regions of shared GMV changes in patients with migraine and RLS. This analysis identified voxels that were significantly different in the relevant contrasts by searching for the intersection of the voxel-wise FWE-thresholded maps from patient–control comparisons (migraine vs. healthy controls and RLS vs. healthy controls). In addition, a direct disease group comparison (migraine vs. RLS) was performed to identify anatomical regions with distinct GMV changes between these two patient groups.

### Replication analysis of shared neural signatures in the additional comorbid migraine/RLS group

We used a two-sample *t*-test to investigate whole brain voxel-wise GMV changes between patients with comorbid migraine and RLS and healthy controls. To further validate the results of the conjunction analysis described in the previous section, we overlapped these two voxel-wise statistical results in the standard MNI-space to check the spatial relationship between the two analyses.

### Relationship between regional GMV and clinical parameters

To investigate the clinical associations in the group of patients with comorbid migraine and RLS, the anatomical regions identified by the conjunction analysis were extracted, averaged, and correlated with clinical characteristics, including migraine duration, migraine frequency, RLS duration, IRLSSG severity score, total PSQI score, Hospital Anxiety and Depression Scale (HADS) score, and Beck depression inventory (BDI) score, using Pearson's correlation. The threshold of statistical significance was set at uncorrected $P < 0.05$.

## Results

### Demographic data and clinical characteristics of the participants

The major clinical characteristics of the participants are presented in Table 1. The four groups were matched for age, gender, and handedness. All patients with migraine who reported aura had visual aura symptoms. Twelve of 22 (54.5%) patients with migraine had a positive family history for migraine. Furthermore, 11 of 27 (40.7%) patients with RLS had a positive family history for RLS; 11 of 22 (50.0%) patients with comorbid migraine and RLS had a positive family history for migraine, and 9 (40.9%) had a positive family history for RLS. There were no significant differences in total GMV, WM volume, CSF volume, or TIV among the four groups. None of the conventional T1 structural images from any participant showed morphological abnormalities or image artifacts.

### Regional GMV changes between migraine patients, RLS patients, and healthy controls

#### Migraine versus healthy controls

GMV differences in patients with migraine relative to healthy controls are shown in Table 2 and Figure 1A (FWE corrected *P* value <0.05). Relative to healthy controls, regions of smaller volume were observed in the left lateral occipital cortex, vermis, and left cerebellum, while regions of greater volume were observed in the right frontal pole and right middle frontal gyrus (MFG).

#### RLS versus healthy controls

Patients with RLS differed from healthy controls in GMV in several regions (Table 2 and Fig. 1B; FWE corrected $P < 0.05$). Relative to healthy controls, regions of smaller volume were observed in the right thalamus, right middle temporal gyrus, right anterior cingulate gyrus (ACC), and left insula; regions of greater volume were observed in the right MFG.

#### Migraine versus RLS (distinct neural signatures of the two disease groups)

Migraine differed from RLS with respect to GMV alterations in several regions (Table 2 and Fig. 2A; FWE corrected $P < 0.05$). Comparing these two patient groups, the migraine patients showed significant GMV increases in the right precuneus, left superior frontal gyrus, and left precentral gyrus, while the RLS patients showed significant GMV increases in the left lateral occipital cortex, left cerebellum and vermis, and left occipital fusiform cortex.

#### GMV alterations common to both disorders (shared neural signature)

Conjunction analysis indicated that in both disorders, the right MFG showed GMV increases compared with the controls (Fig. 2B), while no region showed shared smaller GMV.

 

**Table 1.** Demographic and clinical characteristics of study participants.

| Demographic variables | HC ($n$ = 45) | MIG ($n$ = 22) | RLS ($n$ = 27) | MIG with RLS ($n$ = 22) | $P$ value |
|---|---|---|---|---|---|
| Age (years) | 40.6 ± 10.7 | 36.8 ± 9.9 | 44.5 ± 12.5 | 41.4 ± 12.7 | 0.140 |
| Gender (male/female) | 12/33 | 4/18 | 7/20 | 1/21 | 0.171 |
| Handedness (right/left) | 45/0 | 22/0 | 27/0 | 22/0 | – |
| GMV (c.c.) | 643.5 ± 52.5 | 642.7 ± 58.8 | 617.8 ± 44.4 | 642.5 ± 53.4 | 0.391 |
| WMV (c.c.) | 495.4 ± 45.3 | 484.8 ± 64.3 | 477.8 ± 52.6 | 492.5 ± 43.4 | 0.508 |
| CSFV (c.c.) | 300.7 ± 41.0 | 306.6 ± 50.6 | 311.1 ± 39.3 | 304.1 ± 27.2 | 0.128 |
| TIV (c.c.) | 1439.6 ± 112.9 | 1434.2 ± 144.4 | 1406.7 ± 119.3 | 1439.2 ± 103.5 | 0.229 |
| Aura/no aura | – | 4/18 | – | 5/17 | 0.71 |
| Migraine duration (years) | – | 6.7 ± 7.8 | – | 6.4 ± 6.7 | 0.670 |
| Migraine frequency (days/month) | – | 8.9 ± 4.8 | – | 11.2 ± 9.5 | 0.392 |
| RLS duration (years) | – | – | 10.9 ± 13.4 | 9.4 ± 9.8 | 0.599 |
| IRLSSG severity score (0–40) | – | – | 14.3 ± 7.2 | 13.7 ± 5.2 | 0.773 |
| PSQI total score (0 to −21) | – | 6.9 ± 3.1 | 9.5 ± 2.7 | 11.2 ± 3.6 | 0.001[a] |
| HADS score (0–42) | – | 13.41 ± 8.40 | 12.00 ± 6.37 | 16.11 ± 5.75 | 0.211 |
| BDI score (0 to −63) | – | 9.4 ± 7.8 | 10.3 ± 7.0 | 13.9 ± 8.7 | 0.265 |

Abbreviations: BDI, Beck depression inventory score; CSFV, cerebrospinal fluid volume; GMV, gray matter volume; HADS, Hospital Anxiety and Depression Scale score; HC, healthy controls; IRLSSG, International Restless Legs Syndrome Study Group; MIG, migraine; PSQI, Pittsburgh Sleep Quality Index; RLS, restless legs syndrome; TIV, total intracranial volume; WMV, white matter volume;
[a]Statistically different ($P < 0.05$) with the appropriate statistical test. All patients with migraine or RLS, healthy controls, or patients with comorbid migraine and RLS are right-handed.

**Table 2.** Anatomical regions with significant GMV changes in migraine patients, RLS patients, and healthy controls.

| MNI coordinate $x, y, z$ | Cluster size | Residual GMV mean ± SD | | | Local peak $t$-value | Anatomical region |
|---|---|---|---|---|---|---|
| | | HC | MIG | RLS | | |
| HC > MIG | | | | | | |
| −50, −66, 27 | 655 | 0.007 ± 0.042 | −0.036 ± 0.035 | 0.007 ± 0.048 | 4.48 | Lt. lateral occipital cortex |
| 5, −74, −17 | 584 | 0.011 ± 0.037 | −0.025 ± 0.035 | 0.006 ± 0.038 | 3.71 | Cerebellum vermis |
| −24, −65, −24 | 224 | 0.012 ± 0.052 | −0.028 ± 0.041 | 0.010 ± 0.064 | 3.27 | Lt. cerebellum |
| HC < MIG | | | | | | |
| 20, 44, 38 | 350 | −0.014 ± 0.034 | 0.025 ± 0.034 | 0.000 ± 0.031 | 4.52 | Rt. Frontal pole |
| 48, 29, 38 | 465 | −0.018 ± 0.036 | 0.023 ± 0.046 | 0.006 ± 0.040 | 4.29 | Rt. middle frontal gyrus |
| HC > RLS | | | | | | |
| 14, −30, 11 | 386 | 0.012 ± 0.044 | 0.002 ± 0.050 | −0.023 ± 0.038 | 3.64 | Rt. thalamus |
| 60, −11, −26 | 486 | 0.018 ± 0.047 | −0.006 ± 0.052 | −0.024 ± 0.054 | 3.62 | Rt. middle temporal gyrus |
| 15, 42, 11 | 270 | 0.011 ± 0.039 | 0.000 ± 0.044 | −0.018 ± 0.034 | 3.44 | Rt. anterior cingulate gyrus |
| −62, −12, 9 | 701 | 0.010 ± 0.032 | −0.003 ± 0.028 | −0.022 ± 0.027 | 3.39 | Lt. central opercular cortex |
| HC < RLS | | | | | | |
| 48, 31, 41 | 229 | −0.020 ± 0.037 | 0.018 ± 0.050 | 0.013 ± 0.042 | 3.85 | Rt. middle frontal gyrus |
| MIG > RLS | | | | | | |
| 6, −68, 45 | 287 | −0.004 ± 0.052 | 0.024 ± 0.045 | −0.021 ± 0.043 | 3.91 | Rt. precuneus cortex |
| −15, −8, 72 | 309 | 0.001 ± 0.046 | 0.029 ± 0.050 | −0.019 ± 0.036 | 3.64 | Lt. superior frontal gyrus |
| −44, 8, 20 | 211 | 0.006 ± 0.058 | 0.022 ± 0.046 | −0.024 ± 0.057 | 3.22 | Lt. inferior frontal gyrus |
| RLS > MIG | | | | | | |
| −51, −68, 27 | 434 | 0.003 ± 0.042 | −0.038 ± 0.032 | 0.012 ± 0.046 | 5.37 | Lt. lateral occipital cortex |
| 0, −74, −21 | 383 | 0.006 ± 0.038 | −0.022 ± 0.034 | 0.014 ± 0.038 | 3.51 | Cerebellum vermis |
| −32, −63, −15 | 289 | 0.004 ± 0.048 | −0.027 ± 0.032 | 0.016 ± 0.047 | 3.50 | Lt. temporal occipital fusiform cortex |

Abbreviations: GMV, gray matter volume; HC, healthy controls; Lt, left; MIG, migraine; MNI, Montreal Neurological Institute; RLS, restless legs syndrome; Rt, right; SD, standard deviation. Peak of group differences in residual GMV with a threshold of threshold of FWE-corrected $P < 0.05$.

 

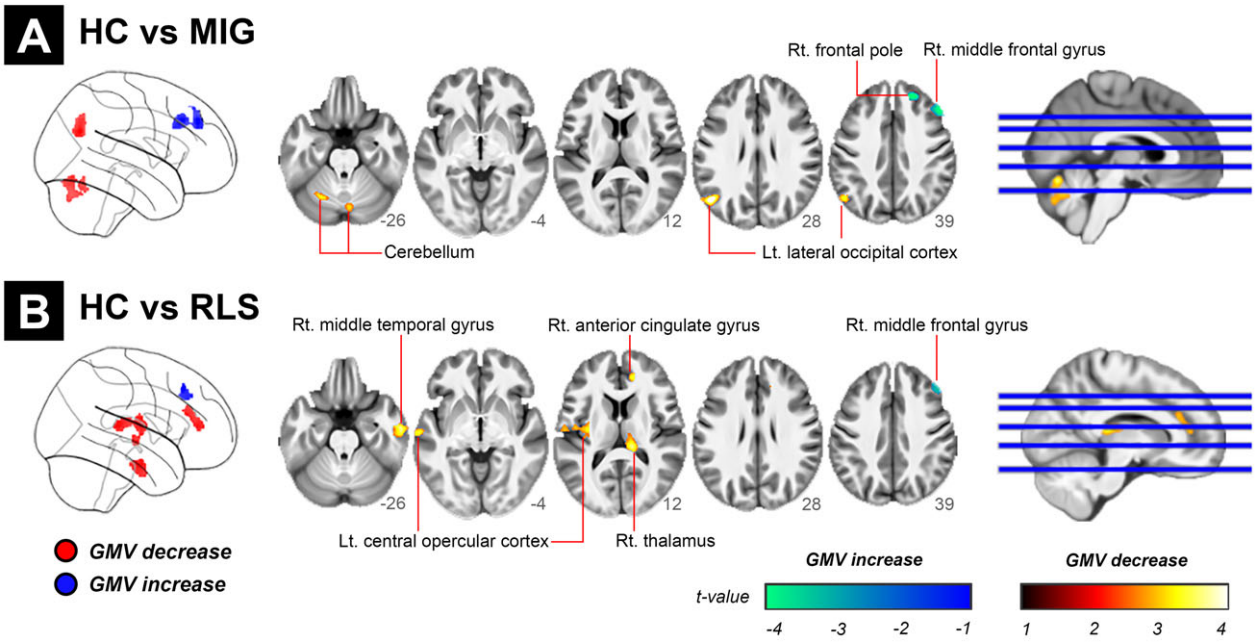


**Figure 1.** (A) GMV differences between migraine patients and the healthy control group as revealed by VBM. Cold (blue) colors significantly greater regional GMV in patients with migraine; warm (red) colors indicate significantly lower regional GMV. (B) GMV differences between RLS patients and the healthy control group. The cold colors indicate a significantly greater regional GMV in patients with RLS. Abbreviations: GMV, gray matter volume; HC, healthy controls; Lt, left; MIG, migraine; RLS, restless legs syndrome; Rt, right; VBM, voxel-based morphometry.

### Patients with comorbid migraine and RLS versus healthy controls (the shared pattern of neural signature replicated in this comorbidity dataset)

GMV differences in patients with comorbid migraine and RLS relative to healthy controls are shown in Table 3 and Figure 3 (FWE corrected $P < 0.05$). Relative to healthy controls, regions of smaller volume were observed in the right cerebellum and right temporal pole, while regions of greater volume were observed in the bilateral MFG and right precentral gyrus. Specifically, altered GMV was consistently found in the right MFG in patients with comorbid migraine and RLS relative to controls.

### Associations between clinical variables and regional GMV changes

In patients with comorbid migraine with RLS, the sleep quality as assessed using the PSQI score correlated negatively with the GMV of the right MFG ($r = -0.492$, $P = 0.038$).

## Discussion

In the present study, compared with healthy controls, migraine patients showed GMV changes in the lateral occipital cortex, cerebellum, frontal pole, and MFG, whereas RLS patients showed GMV changes in the thalamus, middle temporal gyrus, ACC, insular, and MFG. Several regions, including the precuneus, superior frontal gyrus, precentral gyrus, lateral occipital cortex, cerebellum and vermis, and occipital fusiform cortex, differed between the two disorders, indicating that each is associated with a spatially distinct pattern of pathophysiology. Our conjunction analysis also demonstrated that the two disorders share similar patterns of GMV changes in the MFG. Alterations in the GMV of this area were consistent in patients with comorbid migraine and RLS. Finally, we observed that the GMV of the MFG demonstrated a significant association with sleep quality.

Both migraine and RLS demonstrated several areas with GMV alternations relative to control groups. Migraine patients show increased sensitivity to light, which may be related to changes in cortical responsiveness involving lack of habituation to repeated visual stimulation, or to deficits in visual processing.[26] Studies have demonstrated that tonic pain can induce electroencephalographic and functional imaging changes in the occipital regions.[27] Therefore, the GMV changes in the occipital cortex may be related to visual hyperexcitability, or to changes in cerebral metabolism or neurotransmitter composition.[28] Previous research has suggested that the cerebellum and the deep cerebellar nuclei are related to trigeminal nociception and pain modulation.[29] In

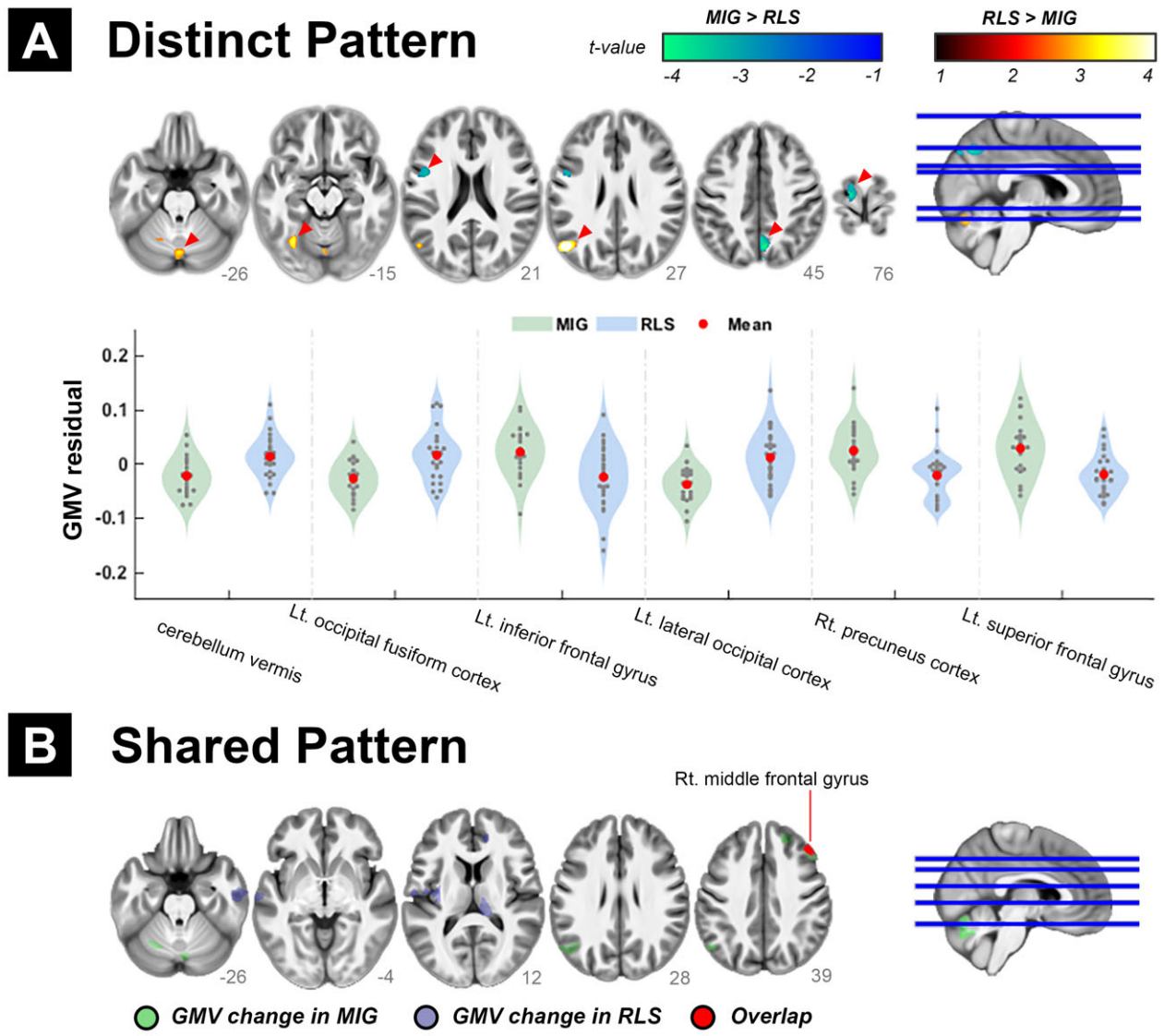


**Figure 2.** (A) GMV differences between migraine patients and the RLS patients as revealed by VBM. Cold (blue) colors indicate a significantly greater regional GMV in migraine patients; hot (red) colors indicate a significantly smaller regional GMV. (B) GMV alterations common to both migraine and RLS patients. Abbreviations: GMV, gray matter volume; Lt, left; MIG, migraine; RLS, restless legs syndrome; Rt, right; VBM, voxel-based morphometry.

concordance with a previous study,[30] we found altered GMV in migraine patients in regions of the frontal lobes (frontal pole and MFG) that are thought to be part of the pain-processing network and are also involved in executive functions.[31] Collectively, these GMV alterations may help to explain the interictal deficits of migraine patients in visual motion processing, pain modulation, and executive functions.

We found that patients with RLS showed GMV alterations in thalamus, middle temporal gyrus, ACC, insula, and MFG. The involvement of the thalamus accords with previous research indicating that GMV alterations of thalamic structures may be associated with the pathogenesis of RLS.[32] The temporal gyrus is connected with the frontal cortices (including MFG) and insula and is involved in pain perception and processing.[33] RLS pathogenesis was previously thought to be related to dysfunction of the sensorimotor and limbic systems, of which the ACC is an important component.[34] These functions may be related to the GMV changes between RLS patients and controls observed in this study, suggesting disruptions of these sensory-related networks causing abnormal sensory-motor integration and abnormal modulation of neural responses to aversive stimuli.

 

**Table 3.** Anatomical regions with significant GMV changes in patients with comorbid migraine and RLS compared with the healthy control group.

| MNI coordinate *x*, *y*, *z* | Cluster size | Residual GMV mean ± SD | | Local peak *t*-value | Anatomical region |
|---|---|---|---|---|---|
| | | HC | MIG with RLS | | |
| HC > MIG with RLS | | | | | |
| 20, −43, −50 | 437 | 0.012 ± 0.036 | −0.023 ± 0.034 | 4.52 | Rt. cerebellum |
| 32, 5, −20 | 294 | 0.013 ± 0.034 | −0.014 ± 0.024 | 3.48 | Rt. temporal pole |
| HC < MIG with RLS | | | | | |
| −39, 2, 59 | 966 | −0.014 ± 0.040 | 0.030 ± 0.038 | 4.05 | Lt. middle frontal gyrus |
| 57, −3, 50 | 227 | −0.013 ± 0.045 | 0.028 ± 0.051 | 3.37 | Rt. precentral gyrus |
| 45, 32, 29 | 489 | −0.014 ± 0.033 | 0.016 ± 0.028 | 3.35 | Rt. middle frontal gyrus |

Abbreviations: GMV, gray matter volume; HC, healthy controls; Lt, left; MIG, migraine; MNI, Montreal Neurological Institute; RLS, restless legs syndrome; Rt, right; SD, standard deviation. Peak of group differences in residual GMV with a threshold of threshold of FWE-corrected $P < 0.05$.

One of the main findings of the present study concerns the direct comparison between migraine and RLS. There are fundamental distinctions between the clinical presentations of migraine and RLS; therefore, marked differences in their neural structural signatures are also expected. To date, only a limited number of brain imaging studies have compared these conditions, either directly or indirectly.[20] Our previous functional imaging study reported FC differences in the salience, default mode to subcortical and frontoparietal, auditory to salience, and memory retrieval networks between migraine patients with and without RLS.[20] However, it remained unclear whether these functional changes were associated with structural changes, and no patients with RLS only were examined. Furthermore, the comparison between migraine and RLS may help disclose regions with structural changes that are associated with clinical differences and spatially distinct patterns for each pathophysiology.

In this study, the comparison between migraine and RLS groups revealed GMV differences in the precuneus, superior frontal gyrus, precentral gyrus, lateral occipital cortex, cerebellum (vermis), and occipital fusiform cortex. We found several new regions that have not been reported previously in patients with migraine or RLS compared with healthy individuals, such as the superior frontal and precentral gyri, precuneus and occipital fusiform cortex, and vermis. These regions showed changes in GMV and are related to the frontal, occipital, and cerebellar networks, which are associated with nociceptive modulation and visual and motor processing. Thus, these differences may be associated with altered nociceptive control of sensory inputs and abnormal sensory-motor integration, resulting in clinical differences and spatially distinct patterns of pathophysiology between migraine and RLS.

Conjunction analysis further indicated that the GMV of the MFG was robustly increased in both migraine and RLS groups. Frontal structural (including MFG) changes are associated with headache frequency in patients with episodic and chronic migraine.[35] MFG structural changes are also associated with executive function in patients with migraine.[36] Furthermore, patients with RLS have discernable brain structural alterations in the medial frontal lobe (including MFG).[32] MFG activation was also found in patients with RLS during active plantar flexion and dorsiflexion of both feet, suggesting an association with long-standing RLS symptoms.[37] Additionally, the MFG is proposed as an area of convergence for attention networks that interrupts ongoing endogenous attentional processes and reorients attention to an exogenous stimulus.[38] The MFG may also be associated with pain modulation and inhibitory control.[39] Collectively, brain imaging evidence suggests that the pathogenesis of both migraine and RLS may be associated with dysfunction and modulation of nociceptive perception.[20,34] Therefore, the MFG may be critically involved in the comorbidity of migraine and RLS, although future research is warranted.

Our study further highlighted that altered GMV relative to controls was consistently found in the MFG in patients with comorbid migraine and RLS. Additionally, our correlation analyses revealed that GMV in the MFG was negatively correlated with PSQI in patients with comorbid migraine and RLS. In other words, the better the sleep quality, the larger the GMV of the MFG in patients with comorbid migraine and RLS. Previous studies have reported that chronic primary insomnia patients showed significant GMV reduction in the prefrontal cortices (including MFG),[40] and a prior functional imaging study demonstrated that lower regional homogeneity of brain activity in the frontal gyrus (including MFG) in chronic primary insomnia patients than in sound sleepers.[41]

 

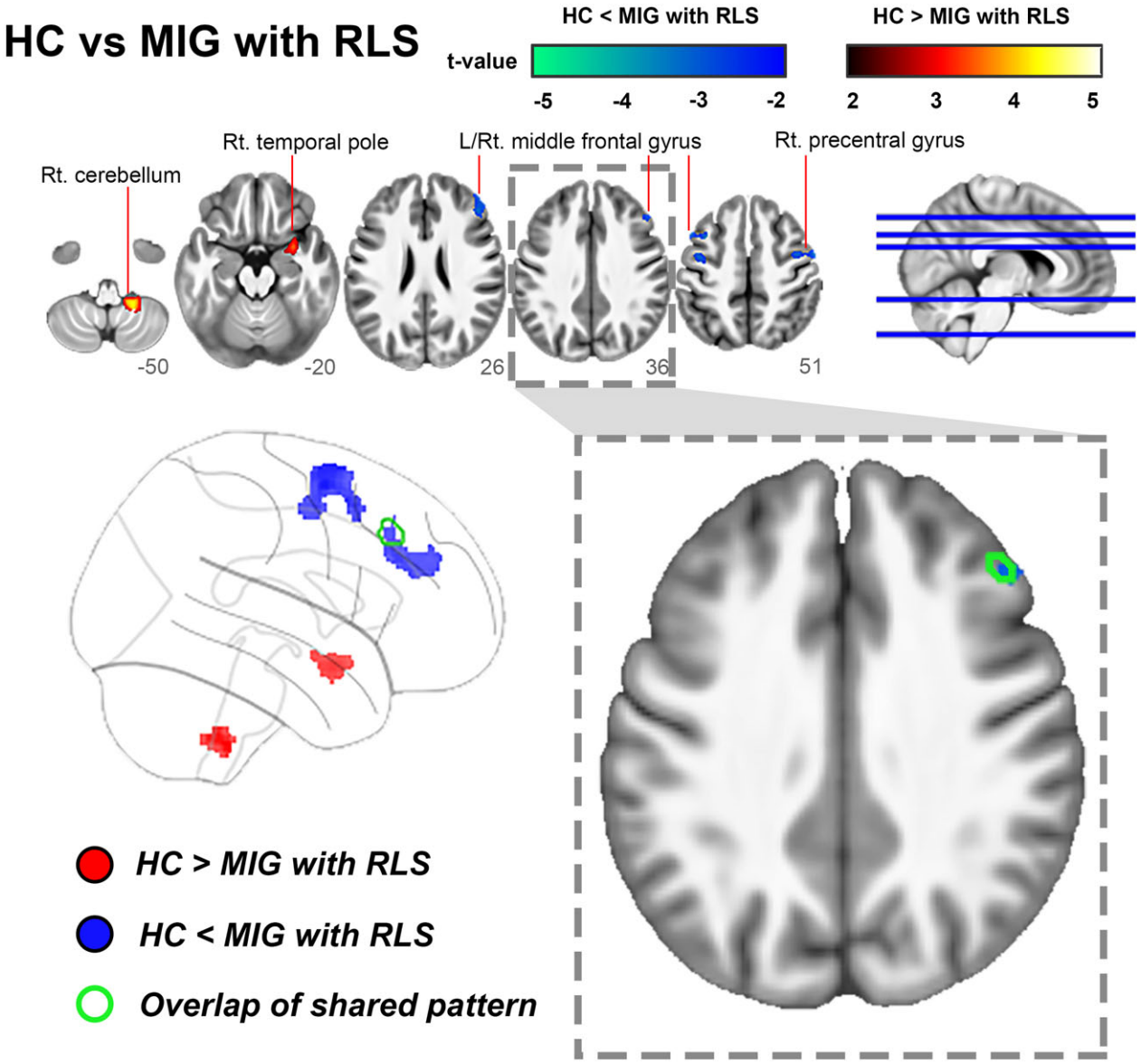


**Figure 3.** GMV differences between patients with comorbid migraine and RLS and the healthy control group as revealed by VBM. Cold (blue) colors indicate a significantly greater regional GMV in patients with comorbid migraine and RLS; hot (red) colors indicate a significantly smaller regional GMV. Abbreviations: GMV, gray matter volume; HC, healthy controls; Lt, left; MIG, migraine; RLS, restless legs syndrome; Rt, right; VBM, voxel-based morphometry.

Furthermore, evidence suggests that migraine and RLS are associated with a high frequency of poor sleep.[7,42] Considering these factors, we speculate that the GMV of the MFG is a potential biomarker for indexing sleep quality in patients with comorbid migraine and RLS. Collectively, the consistent finding of GMV alterations in the MFG reinforces the conclusion that the GMV alternations of the MFG may be related to the shared pathophysiology of these two disorders. Further work is necessary to confirm this hypothesis.

To our knowledge, this study is the first to investigate structural alterations in migraine patients, RLS patients, and patients with comorbid migraine and RLS. However, our study had several limitations. First, the sample size was modest, although we used a strict definition for well-characterized diagnoses, which may decrease subject variability and ensure optimum detectability of group differences. Nevertheless, our preliminary/exploratory findings can be used to guide future large-scale investigations, and future studies with larger samples are warranted to

 

replicate and extend the present results. Second, we used a cross-sectional group comparison design in this study; therefore, we could not determine causality. This means that it is not clear whether the observed alterations are part of the pathogenesis or consequences of these disorders. Future longitudinal studies may be required to determine whether the structural alterations are dynamic. Third, we used the statistical criteria with conservative Bonferroni corrected $P < 0.05$ in this study; however, we did not find any statistically significant results for our additional clinical correlation analyses. Using this approach may lower the Type I error, but it may increase Type II error rates. Therefore, to shed some light on the potential role of MFG in the pathophysiology of migraine and RLS comorbidity, we chose to report the final result with a more liberal statistical threshold of $P < 0.05$ (uncorrected). Finally, because in the present study most brain areas exhibiting GMV alteration were associated with higher cortical functions, future studies should employ cognitive testing (i.e., attention, execution, or visual) to better understand the relationships between these brain GMV alterations and cognition.

In conclusion, in this study, we observed altered GMV in patients with migraine, RLS, and comorbid migraine and RLS. The migraine and RLS groups exhibited distinct patterns of GMV alterations, predominantly in the frontal, occipital, and cerebellar areas. Both groups also displayed a common pattern of GMV changes predominantly affecting the MFG. Additionally, altered GMV in the MFG was consistently found in patients with comorbid migraine and RLS, and the GMV of this area also associated with sleep quality. Therefore, these findings suggest a potential anatomical signature related to the shared pathophysiology of these two disorders.

## Acknowledgments

The authors acknowledge the MRI of Tri-Service General Hospital, National Defense Medical Center, Taiwan for their assistance with the MRI experiments. This project was supported in part by grants from the Ministry of Science and Technology of Taiwan (MOST 105-2314-B-016-004-, MOST 106-2314-B-016 -007 -MY2, MOST 106-2221-E-010-011, MOST 107-2221-E-010-010 -MY3, MOST 106-2221-E-010-011-); Tri-Service General Hospital, Taiwan (TSGH-C101-159, TSGH-C106-068, TSGH-C107-072).

## Author Contribution

Study concept/design: F.C.Y., K.H.C., P.L.L., C.P.L., J.T.L.; data collection and analysis: F.C.Y., K.H.C., P.L.L., C.P.L., J.T.L.; data interpretation: F.C.Y., K.H.C., P.L.L., J.H.Y., S.Y.C. H.W.K., Y.F.S., C.H.C., C.K.T., C.L.T., C.P.L., J.T.L.; manuscript writing: F.C.Y., K.H.C., P.L.L., C.P.L., J.T.L.

## Conflict of Interest

The authors declare that they have no conflicts of interest.

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