Multimodal Characterization of Grey Matter Alterations in Neuromyelitis Optica

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Abstract

Objective: To investigate structural and functional alterations of gray matter (GM) and examine their clinical relevance in neuromyelitis optica (NMO) using multimodal MRI techniques.

Methods: Thirty-five NMO and 36 healthy controls (HC) were recruited in this study. Cortical lesions were investigated by double inversion recovery (DIR) technique. Five voxel-wised MRI measurements were obtained for each participant in the GM including GM volume (GMV), fractional anisotropy (FA), mean diffusivity (MD), amplitude of low-frequency fluctuation (ALFF), and weighted functional connectivity strength (wFCS). Between-group differences, cross-modality relationships and MRI-clinical correlations were examined.

Results: No cortical lesions were found in NMO. Compared to HC, NMO patients exhibited significantly decreased GMV in deep GM and cortical regions involving visual function and cognition. Diffusion GM abnormalities were widespread in the patients. Decreased ALFF and wFCS were observed in the patients in sensorimotor, visual, cognition and cerebellar sites. GM structural alterations were correlated with cognitive but not physical disability scores of the patients.

Conclusion: Despite the lack of focal cortical lesions, patients with NMO exhibit both structural and functional alterations of GM in cerebrum and cerebellum that predominantly involve deep GM, visual, motor and cognitive regions. GM alterations are associated with cognitive impairment but not physical disability.

Keywords: neuromyelitis optica; grey matter; structural MRI; diffusion tensor imaging; resting-state functional MRI

Abbreviations: ALFF = amplitude of low-frequency fluctuation; EDSS = expanded disability status scale; FA = fractional anisotropy; GMV = gray matter volume; MD = mean diffusivity; MRI = magnetic resonance imaging; NMO = neuromyelitis optica; PASAT = paced auditory serial addition test; rs-fMRI = resting-state functional MRI; wFCS = weighted functional connectivity strength

Introduction

Neuromyelitis optica (Devic's disease) (NMO) is an astrocytopathy and demyelinating disease of central nervous system (CNS), typically without brain lesions on conventional MR images, especially in early disease[1, 2]. However, recent neuroimaging studies consistently identify MRI abnormalities in NMO such as periependymal lesions surrounding the ventricular system, white matter lesions, and lesions involving corticospinal tracts[3]. Moreover, advanced MRI techniques have demonstrated cerebral abnormalities[4-7], especially gray matter (GM) alterations[8-11], which may be the neural basis of cognitive impairments in NMO.

In NMO, cortical lesions were reported to be absent using double inversion recovery (DIR) sequence[8]. However, using structural and diffusion MRI, both GM atrophy[9, 12] and microstructural abnormalities[13, 14] were separately identified. Moreover, functional MRI studies revealed GM abnormalities in both task-evolved[15] and spontaneous brain activity[6, 16]. These studies advance our understanding of GM pathology in NMO, however, most of the above findings are from a single modality with differences in samples, clinical features and grouping criteria. Relative to a single modality, recent studies have highlighted the importance of multimodal integration in revealing neural substrate underlying various brain disorders[17, 18]. In NMO, there are two prior multimodal studies. One investigated structural alterations in a small sample (n=8)[19], the other focused on the thalamus[20].

In the current study, we systematically investigated structural and functional alterations of GM in NMO by combining DIR, high-resolution anatomical MRI, diffusion and resting-state functional MRI (rs-fMRI). Furthermore, we examined the similarities and differences among different MRI modalities in the locations of

NMO-related abnormalities. Finally, clinical correlates of GM structural and functional alterations were examined.

Materials and methods

Participants

This study was approved by institutional review board of Xuanwu Hospital, Capital Medical University and written informed consent was obtained from each participant. Forty NMO patients were enrolled in this study from January 2012 to December 2013. NMO diagnosis was made according to the 2006 diagnostic criteria[2]. The demographic information and clinical characteristics including Expanded Disability Status Scale (EDSS)[21], disease duration, number of relapses, mini-mental state examination (MMSE), paced auditory serial addition test 2 seconds version (PASAT 2) and paced auditory serial addition test 3 seconds version (PASAT 3) were recorded for all the patients. All the patients' clinical attacks were restricted to spinal cord and optic neuritis. All the patients were relapsing NMO and twenty-seven patients (27/40, 67.5%) were AQP4 antibody positive measured by an indirect immunofluorescence method[22]. All the patients received steroid treatment, and seven patients received additional immunosuppressant therapy. We enrolled 37 gender-, age-, and education-matched healthy controls (HC) with no previous history of neurological or psychiatric dysfunction and with normal findings on neurological and MRI examination. Of note, dataset from a subset of the general population have been used to study the thalamus in patients with NMO[20].

Image acquisition

All the multimodal MRI scans including conventional MR sequences (T2 and FLAIR), double inversion recovery (DIR), high-resolution T1WI, diffusion tensor imaging (DTI), resting-state fMRI were performed on a 3.0 Tesla MR system (Siemens Magnetom Trio Tim system, Germany). The MRI scans of the NMO patients were acquired at least 2 weeks from the last relapse and treatment to minimize their confounding effects on subsequent multimodal brain measurements. The median time from the last relapse of the patients is 7 months with a range from 2 to 11 months. See appendix e-1 for detailed multimodal MR imaging parameters.

Multimodal image analysis

Based on the multimodal MRI data acquired above, we analyzed WM lesion from T2-weighted and FLAIR images, GM lesion from DIR images, GM volume from T1 images, microstructural integrity (fractional anisotropy, FA and mean diffusivity, MD) from diffusion images, and intrinsic functional architecture including amplitude of low-frequency fluctuation, ALFF (local oscillation power)[23] and weighted functional connectivity strength, wFCS (inter-regional functional connectivity over the whole brain)[24] from rs-fMRI. For details of multimodal imaging preprocessing and measure calculation, see appendix e-1.

Statistical analysis

Discrete gender data were analyzed using a chi-square test. For other demographic and clinical continuous variables, two-sided two-sample t test or Wilcoxon rank sum tests were used to assess between-group differences depending on whether they were normally distributed (Lilliefors test). For the multimodal MRI measures, a voxel-wise general linear model was used to infer between-group differences. To correct for the multiple comparisons, a height threshold of $p < 0.001$ combined with an extent threshold of $p < 0.05$ were employed, which corresponded to a corrected $p < 0.05$ as determined by the Monte Carlo simulations[25]. Dice coefficient and partial correlation analyses were used to estimate cross-modality spatial overlap and quantitative relationships for regions showing NMO-related alterations. Partial correlation was also used to estimate clinical-MRI relationships in the patients. The effects of age and gender were controlled for all MRI-related statistical analyses (i.e., between-group comparison and correlation analysis). Gross and subtle head motion parameters were treated as extra covariates for rs-fMRI-based analysis. Additionally, we also examined effects of clinical attacks (optic neuritis or myelitis) and white matter lesions on multimodal MRI alterations in the patients (appendix e-1).

Results

Demographic and clinical characteristics

For the current dataset, two NMO patients exhibited relatively large cortical defects possibly associated with either old infarcts or trauma, and thus were excluded conservatively from further analyses. In addition, three NMO patients and one HC

were excluded due to image artifacts by visual inspection. All the demographic and clinical characteristics for the remaining 35 NMO patients and 36 HC are summarized in Table 1. There were no significant differences in age, gender and educational level between the NMO and HC groups (all $p > 0.05$). The patients had significant lower scores in the PASAT 2, PASAT 3 and MMSE compared with the HC (all $p < 0.05$). The cognitive deficits remained significant in NMO after taking educational level as a covariate.

WM and GM lesions

Twenty out of the 35 NMO patients had no WM lesions on cerebral MRI and fifteen had non-specific WM lesions (small rounded WM hyperintensities on T2/FLAIR, which did not fulfill the McDonald criteria for multiple sclerosis lesions). The mean lesion volume was 5.12 (± 3.86) mL for the patients. No cortical lesions were identified in the NMO patients based on DIR.

Between-group differences of morphological and microstructural organization

Compared with the HC, the NMO patients showed significantly decreased GMV in the bilateral thalamus, insula, middle temporal gyrus and anterior cingulate, the right caudate and inferior frontal gyrus, and the left calcarine ($p < 0.05$, corrected) (Figure 1A). For diffusion-based MD and FA, widespread brain regions in both the cerebrum and cerebellum were abnormal compared with the HC. Specifically, in addition to the regions showing decreased GMV, the patients with NMO showed significantly

decreased FA in the precentral gyrus, precuneus and parahippocampal gyrus/hippocampus (all bilateral) and the left amygdala (Figure 1B) and the cerebellar anterior lobe (bilateral, Figure 2A). A similar but even more diffuse pattern of increased MD in the patients compared with the HC was observed. The greatest abnormalities were found in the medial prefrontal, lateral temporal cortex and deep GM structures (Figure 1C and Figure 2B).

Between-group differences of functional measurements

Limited and regionally specific NMO-related alterations were found in the functional scans. Specifically, for the ALFF, significant decreases were mainly observed in sensorimotor (e.g., the bilateral paracentral lobule, the right supplementary motor area and precentral gyrus) and visual cortex (e.g., the right inferior and middle occipital gyrus) ($p < 0.05$, corrected) (Figure 1D), while for the wFCS, NMO-related decreases were primarily observed in visual (e.g., the superior occipital gyrus and cuneus, the right fusiform) and cognition-related areas (e.g., the bilateral middle cingulate gyrus, and the left anterior cingulate gyrus) and the cerebellum ($p < 0.05$, corrected) (Figure 1E and Figure 2C).

Cross-modality relationship

Figure 3A shows spatial overlaps among the five multimodal MRI-based measures in revealing NMO-related GM alterations. The most frequently affected regions included deep GM structures (e.g., the bilateral thalamus and caudate), medial prefrontal cortex

and temporal lobe locations (e.g., hippocampus/parahippocampal gyrus and superior temporal gyrus) and insula. Further pairwise cross-modality comparisons showed that the largest spatial overlap was observed between FA and MD (dice coefficient = 0.289), followed by GMV and MD (dice coefficient = 0.179) and GMV and FA (dice coefficient = 0.087). The spatial overlaps between other combinations were relatively low. Of note, there was no common region that simultaneously exhibited GMV and functional (ALFF/wFCS) alterations in NMO (Figure 3B). We further quantified pairwise cross-modality correlations in the regions (cluster size > 20 voxels) that simultaneously exhibited NMO-related alterations (Figure 3C) in the patient group. Significantly positive correlations were observed between GMV and FA ($r = 0.481$, $p = 0.005$, mean across clusters) and between FA and wFCS ($r = 0.398$, $p = 0.022$, mean across clusters), and significantly negative correlations were observed between GMV and MD ($r = -0.491$, $p = 0.004$, mean across clusters) and between FA and MD ($r = -0.548$, $p < 0.001$, mean across clusters) (Figure 3D).

MRI-clinical relationship

For the NMO patients, significant correlations were observed between structural brain measures (GMV, FA and MD) and clinical characteristics ($p < 0.05$, False Discovery Rate corrected) in multiple GM structures. In detail, the PASAT 2 was correlated with GMV in the left thalamus, FA in the brainstem and MD in the right thalamus; the PASAT 3 was associated with MD in the right parahippocampus and superior temporal gyrus; the disease duration was correlated with FA and MD in the left

parahippocampus/hippocampus; and the number of relapses was related to MD in the bilateral cuneus. The scatter plots of all these correlations are shown in Figure 4. No significant correlations were observed between the EDSS (including cerebellar functional system score) and the multimodal MRI measurements in the patients. There were also no significant effects of clinical attacks (optic neuritis or myelitis) and white matter lesions on the multimodal MRI measurements in the patients ($p > 0.05$, False Discovery Rate corrected).

Discussion

In the current study, we examined GM structural and functional alterations in NMO by employing multimodal quantitative MRI techniques. The main findings can be summarized as: 1) no focal cortical lesions were found in NMO patients; 2) both structural and functional alterations in GM were identified in NMO, with structural abnormalities being much more extensive than functional changes. Predominantly affected areas included deep GM nuclei and cortical regions involved in vision, motor function and cognition; 3) multimodal MRI provided complementary information by capturing NMO-induced GM alterations, with DTI revealing much more widespread abnormalities than other structural and functional MRI measures; and 4) structural GM alterations, particularly microstructural abnormalities, were correlated with the patients' cognitive impairment, but not physical disability.

Nearly half of the included NMO patients showed non-specific WM lesions, while none exhibited cortical lesions in the current study. These findings are

consistent with previous findings[5, 8, 26] and confirm that non-specific WM lesions (not fulfill the criteria for multiple sclerosis lesions) and the absence of cortical lesions is MRI features of NMO distinguishing them from multiple sclerosis[10].

Structural and functional alterations were observed in GM in NMO. Specifically, the VBM analysis revealed regional GM atrophy in multiple regions. This is consistent with previous studies reporting GM atrophy in NMO in terms of either GMV[10-12] or cortical thickness[8, 9]. Specifically, NMO-related GM atrophy principally occurred in deep GM nuclei and several cortical areas related to vision, sensorimotor and cognition such as anterior cingulate cortex. This atrophy could be caused by primary cortical pathology such as an inflammatory-degenerative process induced by Aquaporin-4 (AQP4) or secondary trans-synaptic degeneration triggered by axonal loss in lesions in the optic nerve and spinal cord.

Microstructural abnormalities have been previously reported to be widespread in WM fibers[4, 27]. Using a diffusivity histogram[13] or ROI analysis[14], significant diffusion changes have also been observed in the normal appearing GM in NMO. Our study extends previous findings by using a voxel-based whole-brain method showing that diffusion changes are widely distributed in brain areas implicated in visual, sensorimotor and cognitive functions. Given that a serum immunoglobulin G autoantibody (NMO-IgG) selectively binds to AQP4[28] in NMO, we speculate the disruption of water homeostasis may be one key pathophysiological basis for the observed diffusion changes. As a novel finding, diffusion abnormalities were also found in the cerebellum, possible factors contributing to cerebellar diffusion

abnormalities in NMO include primary pathogenic effect of the anti-AQP4 antibody since AQP4 is highly expressed in cerebellum[29], and secondary degeneration since the cerebellum has direct and dense connections to the spinal cord[30].

To examine functional architecture, we employed ALFF and wFCS to study local activity fluctuations and interregional connectivity in NMO, respectively. Our results showed subtle functional alterations (both for local neural activity [ALFF] and for functional coupling [wFCS]), mainly in motor, vision and cognition related areas, corresponding to the symptoms in NMO, and consistent with previous findings[6, 11]. Interestingly, the cerebellum also showed decreased wFCS in NMO, proposing the cerebellum as a possible NMO-related disconnection site. Further studies are needed to confirm our newly described cerebellar MRI abnormalities.

We observed that most of the brain areas with decreased GMV in NMO were those also harboring diffusion changes, and significant associations were observed between regional FA/MD and GMV in the patients. Given that no focal cortical pathology was observed on the DIR images in the current study and on ultra-high field MR images in previous studies[26], the observed volumetric and microstructural alterations of deep GM and cortical areas may commonly reflect trans-synaptic degeneration caused by axonal loss in the lesions[31]. However, functional MRI changes were partly limited to areas with diffusion abnormalities in the absence of morphological alterations, implying the existence of additional and independent mechanism for functional alterations in NMO. The tight microstructural-morphological and weak structural-functional associations of

multimodal MRI changes in NMO are consistent with our previous studies in multiple sclerosis and/or NMO[20, 32] and suggest that structural and functional fusion analysis could provide unique and complementary information on these two diseases. Notably, there are few studies currently that directly compare these two diseases from a multimodal integrative perspective, which is an important topic in the future. Overall, our results indicate that NMO is associated with common and distinct GM alterations among different MRI modalities and microstructural diffusion abnormalities may be the most sensitive measures to detect early and subtle GM damages in NMO. Future studies are needed to provide deeper insights into these findings by establishing sequential or causal relationships among NMO-related multimodal alterations with the help of computational modeling and simulation.

Significant correlations with cognitive scores of the patients were found for GM structural changes, particularly microstructural abnormalities, in several brain areas, such as thalamus and parahippocampus. This implies that GM structural changes may serve as potential biomarkers for assessing and monitoring cognitive impairment in NMO although longitudinal studies are required. No correlations were observed between physical disability in terms of EDSS and multimodal MRI measurements, suggesting other factors (i.e., spinal cord pathology) rather than brain GM damage account for the largest proportion of physical disability in NMO. Notably, the diffusion abnormalities of cognition related brain areas (e.g., parahippocampus/hippocampus) were correlated with disease duration and cognitive scores, while diffusion alterations in visual areas were correlated to the number of

relapses, indicating different pathological mechanisms underlying GM microstructural damage in NMO. Collectively, these findings highlight a neuropathological and neurocognitive significance of GM changes especially in microstructure in understanding NMO.

The present study had several limitations. First, this was a cross-sectional study, and longitudinal studies are required to assess whether distinct longitudinal changes in GM predict clinical disease progression. Second, the cognitive assessment of the participants only included MMSE and PASAT, which limited a more comprehensive examination of MRI-cognition correlates. A more systematical cognitive evaluation such as Minimal Assessment of Cognitive Function in MS[33] is planned for a future study. Third, all the patients were treated in the current study, and thus we cannot exclude the possibility of treatment effects on our findings of multimodal MRI measures and neuropsychological tests. Future studies will help clarify this issue by exploring brain changes of NMO patients following different treatment strategies or recruiting treatment-naïve patients. Fourth, the current study utilized rs-fMRI to characterize NMO-related alterations of intrinsic functional brain architecture. Considering that NMO is associated with cognitive dysfunction in multiple domains[34], it is interesting in the future to examine functional brain alterations of NMO patients while performing cognitive tasks. Finally, the current study mainly focused on local brain architecture in NMO. Given the highly integrative nature of the brain[35], it will be an interesting topic in future to explore NMO-related cortical reorganization from a network perspective.

In conclusion, despite the lack of focal cortical lesions, patients with NMO exhibited both structural and functional alterations of GM in cerebrum and cerebellum that predominantly involved deep GM, visual, motor and cognitive regions. Multimodal MRI techniques were complementary to capture NMO-related GM abnormalities, but DTI changes were the most widespread. GM alterations, especially diffusion abnormalities were associated with both disease duration and number of relapses, and correlated with cognitive impairment but not physical disability in NMO.

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Disclosures:

Dr. Frederik Barkhof serves as a consultant for Bayer-Schering Pharma, Sanofi-Aventis, Biogen Idec, Teva, Merck Serono, Novartis, Roche, Synthon, and Jansen Research. Dr. Yaou Liu,Dr. Xueyan Jiang, Dr. Helmut Butzkueven, Dr Yunyun Duan, Dr. Jing Huang; Dr. Zhuoqiong Ren, Dr. Huiqiong Dong; Dr. Fu-Dong Shi and Dr. Kuncheng Li report no disclosures.

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Tables and Figures

Table 1. Demographics and clinical characteristics of the participants

	HC (*n*= 36)	NMO (*n*=35)	p-value
Age (years)	30 (21 - 59)	38 (18 - 56)	0.158[a]
Gender (M/F)	8/28	5/30	0.387[b]
Education (years)	14.167 ± 3.385	12.914 ± 3.175	0.113[c]
EDSS	NA	4 (1 - 6)	NA
Disease duration (months)	NA	48.14 ± 45.77 (1 - 180)	NA
Number of relapse	NA	4.43 ± 2.58 (2 - 12)	NA
Time from last relapse (months)	NA	7 (2 - 11)	NA
MMSE	29 (20 - 30)	26 (22 - 30)	< 0.001[a]
PASAT 2	47.44 ± 7.01 (27 - 58)	36.20 ± 9.57 (20 - 56)	< 0.001[c]
PASAT 3	53.58 ± 5.06 (40 - 60)	41.49 ± 8.83 (25 - 60)	< 0.001[c]

Dare presented as mean ± standard (minimum - maximum) or median (minimum - maximum) depending on the normality (Lilliefors test). HC = healthy control; NMO = neuromyelitis optica; M = male; F = female; EDSS = Expanded Disability Status Scale; MMSE = Mini-Mental State Examination; PASAT 2 = paced auditory serial addition test 2 seconds version; PASAT 3 = paced auditory serial addition test 3 seconds version; NA = not available.

[a]The *p*-values were obtained using two-tail Wilcoxon rank sum tests.

[b]The *p*-values was obtained using two-tail Pearson chi-square test.

[c]The *p*-values were obtained using two-sample two-tail t tests.

Figure Legends

Figure 1. Between-group differences in GMV (A), FA (B), MD (C), ALFF (D) and wFCS (E) in the cerebrum.

Abbreviations: ALFF = amplitude of low-frequency fluctuation; GMV = grey matter volume; FA = fractional anisotropy; GMV = gray matter volume; MD = mean diffusivity; wFCS = weighted functional connectivity strength.

Figure 2. Between-group differences in FA (A), MD (B) and wFCS (C) in the cerebellum.

Abbreviations: FA = fractional anisotropy; MD = mean diffusivity; wFCS = weighted functional connectivity strength.

Figure 3. Cross-modality relationships.

A, spatial overlap map among the five measures in revealing NMO-related alterations. B, pairwise dice coefficients in the spatial extent. C, pairwise spatial overlap maps. D, pairwise correlations in the patient group.

*, $P < 0.05$.

**, $P < 0.01$.

***, $P < 0.005$.

Abbreviations: ALFF = amplitude of low-frequency fluctuation; GMV = grey matter volume; FA = fractional anisotropy; MD = mean diffusivity; wFCS = weighted functional connectivity strength.

Figure 4. Relationships between multimodal MRI-based measures and clinical variables.

Abbreviations: CAL = calcarine; FA = fractional anisotropy; GMV = grey matter volume; HIP = hippocampus; MD = mean diffusivity; NR = number of relapse; PASAT = paced auditory serial addition test; PHG = parahippocampus; STG = superior temporary gyrus; THA = thalamus.