Abbreviations

aquaporin 4 seronegative (AQP4-)

aquaporin 4 seropositive (AQP4+)

clinically isolated syndromes (CIS)

isotropic volume fraction (ISOVF)

myelin oligodendrocyte glycoprotein antibody associated disease (MOGAD)

multiple sclerosis (MS)

normal-appearing white matter (NAWM)

neurite density index (NDI)

neuromyelitis optica spectrum disorders (NMOSD)

neurite orientation dispersion and density imaging (NODDI)

orientation dispersion index (ODI)

relapsing-remitting MS (RRMS)

secondary progressive MS (SPMS)

1. Introduction

Multiple sclerosis (MS), neuromyelitis optica spectrum disorders (NMOSD) and myelin oligodendrocyte glycoprotein antibody associated disease (MOGAD) are inflammatory demyelinating diseases which have overlapping MRI and clinical features(Rosenthal et al., 2020). Periventricular white matter hyperintensity (WMH) lesions are common findings, but they only partially account for the cognitive decline and physical disability observed in these disease entities(Cacciaguerra et al., 2019; Filippi et al., 2019). Previous studies have shown that microstructural abnormalities in normal-appearing white matter (NAWM) are associated with disease progression, cognitive impairment and physical disability in MS(Duan et al., 2021; Filippi et al., 2000) .

Recently, a gradient in periventricular microstructural abnormality within NAWM in MS has been demonstrated, which may be explained by several potential external (e.g., a cerebrospinal fluid [CSF] soluble factor, leucocytes entering CSF through damaged blood-brain barrier [BBB] and ependymal processes) and intra-parenchymal pathological mechanisms (e.g., susceptibility to hypoxia of axons and oligodendrocytes in periventricular venous watershed regions, tract-mediated effect of periventricular lesions, and microglial activation)(Brown et al., 2017; Liu et al., 2015). Such a periventricular microstructural abnormality gradient seems to be associated with disease progression: clinically isolated syndromes (CIS) patients with a steeper gradient are more likely to develop MS, and a markedly abnormal gradient is seen in secondary progressive MS (SPMS) compared with relapsing-remitting MS (RRMS).(Brown et al., 2017; Liu et al., 2015; Poirion et al., 2021; Vaneckova et al., 2022) To date, studies on periventricular NAWM abnormality gradients focused on MS.(Brown et al., 2017; Liu et al., 2015; Vaneckova et al., 2022) One study with a small sample size of twenty cases,(Cacciaguerra et al., 2021) suggested an absence periventricular NAWM abnormality gradient in aquaporin 4 antibody seropositive [AQP4+] NMOSD, while there has been no study investigating such a gradient in AQP4 antibody negative [AQP4-] NMOSD or MOGAD. Studies are therefore warranted to investigate whether

AQP4+ NMOSD, AQP4- NMOSD and MOGAD also have gradients in periventricular microstructural abnormalities, and whether this gradient is a distinct feature of MS or a common response of the brain to inflammatory insults.

Periventricular microstructural gradients in NAWM have mainly been seen using magnetization transfer ratio (MTR),(Brown et al., 2017; Liu et al., 2015; Poirion et al., 2021) which correlates with myelin and axonal density, as well as potentially tissue oedema and inflammation.(Gareau et al., 2000; Schmierer et al., 2007) Other studies have demonstrated NAWM gradients using PET by labeling microglia.(Poirion et al., 2021) Diffusion imaging provides additional insights into the type of microstructural abnormalities in NAWM,(Cacciaguerra et al., 2021; De Santis et al., 2019; Sun et al., 2020) and in particular is weighted more towards neuro-axonal densities than myelin when compared with MTR.(Filippi et al., 2012; Schmierer and Miquel, 2018) Multi-shell high angular resolution diffusion imaging (e.g., neurite orientation dispersion and density imaging [NODDI]) can distinguish intracellular and extracellular water diffusion using multicompartmental models, contributing to a more detailed delineation (neurite density and fiber orientation) of both white and grey matter (GM) structure.(Zhang et al., 2012)

Against this background, we conducted a study including MS, NMOSD (both AQP4+ and AQP4-), MOGAD and healthy controls (HCs) aiming to investigate (1) whether MS, NMOSD and MOGAD show common or distinct periventricular microstructural abnormality gradients using advanced diffusion imaging and (2) whether the potential periventricular microstructural abnormality gradients in MS, NMOSD and MOGAD correlate with clinical measures.

2. Methods

2.1 Participants

This study was approved by the institutional review board of Beijing Tiantan Hospital, Capital Medical University, Beijing, China (No. KY 2019-050-02). Written informed consent was obtained from each participant according to the Declaration of Helsinki.

Total of 130 relapsing-remitting MS, 146 NMOSD (112 AQP4+ and 34 AQP4-), 29 MOGAD and 53 age-, sex- and education-matched healthy controls (HCs) were prospectively recruited. Relapsing-remitting MS was determined according to 2017 McDonald criteria, and MS participants were both MOG and AQP4 antibody seronegative.(Brownlee, 2018) NMOSD was diagnosed based on the 2015 International Panel,(Wingerchuk et al., 2015) and included AQP4+ NMOSD and AQP4- NMOSD subgroups according to antibody status (AQP4 antibody detected by cell-based immunofluorescence assay [CBA]).(Takahashi et al., 2006) AQP4- NMOSD patients were MOG antibody seronegative. MOGAD was diagnosed based on international diagnostic recommendations and antibody testing by CBA.(Jarius et al., 2018) Other inclusion criteria for all the patients were (1) a MRI scan four or more weeks from the last attack (to exclude the effect of the acute phase on MRI measures); (2) age between 16 and 65 years (to exclude the potential confounding factors of brain development and aging). Exclusion criteria were: (1) incomplete MRI acquisition; (2) contradictions to MRI or poor image quality; (3) a history of other neurological or neuropsychological diseases (e.g., stroke or dementia). Ultimately, 315 participants including 112 MS, 99 AQP4+ and 28 AQP4- NMOSD, 28 MOGAD and 48 HCs were included in this study (**Table 1** and **Figure 1**).

2.2 Clinical measures

Clinical measures including age, sex, disease duration, Expanded Disability Status Scale (EDSS), number of relapses, treatment information and cognitive scores including Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Symbol Digit Modalities Test (SDMT), California Verbal Learning Test,

Third Edition (CVLT-III), Brief Visuospatial Memory Test-Revised (BVLT-R) and Paced Auditory Serial Addition Test (PASAT) were recorded (**Table 1**).

2.3 MRI acquisition and analysis

2.3.1 MRI acquisition

MR imaging including 3D fluid-attenuated inversion recovery (FLAIR), 3D T1 weighted imaging (T1WI) and multi-shell high angular resolution diffusion imaging was performed using a single 3.0 Tesla MR scanner (Philips Ingenia CX, Best, the Netherlands). Sagittal 3D FLAIR was performed using inversion recovering fast spin echo (IR-FSE) (time of repetition [TR]/time of echo [TE]=4800ms/228ms, time of inversion [TI]=1650ms, flip angle [FA]=90°, image resolution=1mm×1mm×1mm, slice number=196); Sagittal 3D T1W images were acquired using magnetization-prepared rapid gradient echo (MPRAGE) (TR/TE=6.6ms/3ms, TI=880ms, FA=8°, image resolution=1mm×1mm×1mm, slice number=196). Axial multi-slice multi-shell high angular resolution diffusion images were acquired using spin echo-echo planar imaging (SE-EPI) (TR/TE=4000ms/88ms, FA=90°, image resolution=2.5mm×2.5mm×2.75mm, slice number=60, b values=0, 1000, 2000 s/mm^2, number of the non-zero diffusion sensitive gradient directions=48), which is a routine protocol suitable to NODDI fitting.(Jelescu et al., 2015; Kimura et al., 2019)

2.3.2 WMH and brain tissue segmentation

WMH were segmented using Lesion Segmentation Tool (LST, version 3.0.0, https://www.applied-statistics.de/lst.html) using T1W and FLAIR images, and further checked by an experienced neuroradiologist (Y.D. with 14 years' experience in neuroradiology).

Segmentations of WM, GM and CSF were performed on lesion-filled T1W images by Computational Anatomy Toolbox (CAT) in Statistical Parametric Mapping (SPM12) and normalized into Montreal Neurological Institute (MNI) space.(Ashburner and Friston, 2005) Total intracranial, WM and GM volumes (TIV, WMW, GMV

respectively) were extracted. WMH frequency maps were calculated (**Figure 2**).(Cao et al., 2020)

2.3.3 Diffusion image processing and NODDI metric calculation

Multi-shell high angular resolution diffusion images were preprocessed using FMRIB Software Library (version 6.0) including eddy-current and motion artifact correction, and skull removal.(Smith et al., 2004) NODDI model fitting was performed using Accelerated Microstructure Imaging via Convex Optimization (AMICO, https://github.com/daducci/AMICO). NODDI metrics including neurite density index (NDI), orientation dispersion index (ODI) and free water fraction (isotropic volume fraction [ISOVF]) mapping were obtained.(Daducci et al., 2015)

For each subject, the B0 image was first co-registered to 3D T1W images by affine transformation. Then all the diffusion images were warped to MNI space using the transformation matrices of B0 to 3D T1WI and 3D T1WI to MNI space (forward transformation in CAT segmentation). Then, all the following processing were conducted in MNI space unless otherwise specified.

2.3.4 Ventricular mask creation

The following steps were conducted to obtain the individual ventricular mask (including lateral and third ventricles): (1) The segmented CSF masks of all subjects were averaged to obtain a population-level CSF map; (2) a population-level ventricle mask (including lateral and third ventricles) was manually delineated by a senior neuroradiologist (Y.D.) based on the population-level CSF map; (3) individual-level CSF masks (threshold>0.5), and a population-level ventricle mask, were binarized (threshold>0); (4) the overlapping area of the binarized individual-level CSF mask and population-level ventricle mask was defined as the individual ventricle mask.

2.3.5 NAWM mask creation

WM masks were defined by binarizing the segmented WM (including both NAWM and

WMH) with a threshold>0.9 to avoid partial volume influence of CSF and GM.(Liu et al., 2015) The NAWM were defined by subtracting the dilated WMH segmentation (dilated by two voxels in all directions) from the WM mask, similar to a previous work, to avoid the partial volume influence of WMH.(Liu et al., 2015)

2.3.6 Segmentation of WM into concentric periventricular rings

For each subject, the distance from ventricle of each voxel in NAWM and undilated WMH mask was defined as the nearest Euclidean distance to the ventricular mask. Then, each 3mm thick band (corresponding to two-voxels in MNI space and approximate to the diffusion imaging resolution) was defined as a concentric periventricular ring. The ring closest to the ventricle was excluded to avoid the potential influence of CSF, and following ten rings were studied.

2.3.7NODDI metric and WMH volume extraction within each ring

The raw NODDI metrics in NAWM were extracted and averaged within each concentric ring. For NAWM rings, a normalized NODDI metric (z-score=[NODDI metric - mean value of NODDI metrics in HCs] / [standard derivation of NODDI metric in HCs]) in patients was calculated and used to represent the alteration of NODDI metric relative to that in HCs, which was independent of the intrinsically heterogeneous raw NODDI metrics across rings.(Vaneckova et al., 2022)

Additionally, the WMH volume and raw NODDI metrics (based on undilated WMH segmentation) within each ring were extracted and normalized NODDI metrics within WMH rings were obtained relative to those in NAWM ring of HCs as above. The raw NODDI metrics within the whole brain NAWM and WMH were extracted and averaged (**Table 1**).

2.4 Statistical analyses

Statistical analyses were conducted using SPSS software (Version 22; SPSS, IBM) and Matlab Statistics and Machine Learning Toolbox (MATLAB 2019a; Mathworks).

Categorical data are displayed by percentage. Continuous and ranked data are displayed as median and interquartile range (IQR). Categorical data between groups were analyzed using chi-square test. Continuous and ranked data were analyzed using one-way analysis of variance (ANOVA) or Kruskal-Wallis tests followed by post-hoc comparison with Bonferroni correction.

Linear mixed models (LMMs) were used to evaluate the relationship between MRI metrics (e.g., normalized NODDI metrics) and distance from the ventricle for NAWM and WMH, respectively. The MRI metrics in each ring were treated as the dependent variable and the following variables were used as independent variables including fixed effect for age, sex, and ring distance from the ventricle to cortex, and random effect for intercept and ring distance from the ventricle to cortex in each group (MS, AQP4+ NMOSD, AQP4- NMOSD, MOGAD and HC) and individuals.

In these models, the relationship between the MRI metric and the ring distance from ventricle to cortex was described by the intercept and slope parameters of a linear model: (1) the model intercept represents the estimated MRI metric in first periventricular ring; (2) the model slope reflects the rate of change in the MRI metric along the distance from the ventricle (periventricular abnormality gradient). Both parameters (intercept and slope) were estimated as linear combinations of model coefficients in fixed and random effects for individuals. The subsequent group- and individual-level analyses were based on these two parameters.

For each model at group-level, the significances of estimated MRI metric in the first periventricular ring and periventricular abnormality gradient in each group were tested using one-sample t-test and differences between patients and HCs were tested using ANOVA and post-hoc t-test with Bonferroni correction.

Linear regressions were conducted to investigate the relationship of periventricular abnormality gradient in NAWM with WMH volume, WMV, GMV, disease duration,

number of relapses, EDSS and cognition scores, with age and sex as covariates. To exclude the potential influence of the whole brain NAWM damages on the clinical associations of periventricular abnormality gradient, we further used whole brain NAWM NODDI metric as an additional covariate. WMH volume, disease duration, number of relapses and EDSS were log-transformed due to their non-normal distribution. For WMV and GMV, TIV was used as an additional covariate.

Statistical significance was defined as two-sided $p<0.05$ as the exploring nature of this study.

3. Results

3.1 Whole brain alterations of NAWM NODDI metrics

Whole brain NAWM-NDI was lower in MS (median value= 0.58) compared to AQP4+ NMOSD (median value=0.59, p=0.007 using ANOVA post-hoc t-test), AQP4- NMOSD (median value=0.60, p<0.001) and HCs (median value=0.60, p<0.001). Whole brain NAWM-NDI was lower in AQP4+ NMOSD compared to HCs (p=0.031). No differences in whole brain NAWM-ODI or NAWM-ISOVF were observed among groups (**Table 1**).

3.2 Periventricular gradients of normalized NAWM NODDI metrics

For normalized NODDI metrics in first periventricular ring, lower normalized NAWM-NDI and NAWM-ISOVF, and higher normalized ODI were observed in MS (average LMM regression intercept=-2.57, -2.47, 0.13) compared to AQP4+ NMOSD (intercept=-1.09, -0.94, -0.76, all p<0.05 using ANOVA post-hoc t-test), AQP4-NMOSD (intercept=-0.27, -0.74, -0.92, all p<0.05), MOGAD (intercept=-1.14, -1.03, -0.80, all p<0.05) and HCs (intercept=-0.034, -1.03, -1.06, all p<0.05). No differences among other groups were observed (**Table 2**).

Significant periventricular abnormality gradients in normalized NAWM-NDI (average LMM regression slope=0.11, p<0.001 using one-sample t-test), NAWM-ODI (slope=-0.11, p<0.001), NAWM-ISOVF (slope=0.20, p<0.001) at group-level were observed in MS but not in AQP4+ NMOSD, AQP4- NMOSD or MOGAD (**Table 2** and **Figure 3**).

3.3 Periventricular gradients of WMH metrics

Total WMH volumes were larger in MS (median value=11.99 ml) compared to AQP4+ NMOSD (median value=2.95 ml, p<0.001 using ANOVA post-hoc t-test), AQP4-NMOSD (median value=2.20 ml, p<0.001), MOGAD (median value=3.35 ml, p=0.003) and HCs (median value=1.71 ml, p<0.001). The findings in first periventricular ring between groups were similar to those of total WMH volumes. Statistically significant

periventricular WMH volume gradients were observed in MS (average LMM regression slope=-0.063, p<0.001 using one-sample t-test), AQP4+ NMOSD (slope=-0.019, p=0.002), MOGAD (slope=-0.036, p=0.033) and HCs (slope=-0.013, p=0.013), but not in AQP4- NMOSD (slope=-0.0094, p=0.085). Additionally, lower WMH- NDI, ODI and ISOVF were observed in MS compared to AQP4+ (except for WMH-NDI) and AQP4- NMOSD, MOGAD and HCs (all p<0.05). Statistically significant periventricular gradients in normalized WMH- NDI, ODI and ISOVF were observed in all groups (except for normalized WMH-ODI in MOGAD and HCs, details were found in **Table 1**, **Table 2** and **Figure 3**).

3.4 Associations of individual periventricular NAWM abnormality gradients with brain tissue volumes and clinical measures

Associations of the periventricular abnormality gradient of normalized NAWM NODDI metric estimated at individual-level with different tissue compartments (WMH volume, WMV and GMV) and clinical measures (disease duration, number of relapses, EDSS and cognitive scores) were examined for MS, AQP4+ NMOSD, AQP4- NMOSD and MOGAD respectively (**Figure 4 and 5**).

For MS, the periventricular normalized NAWM-NDI abnormality gradient correlated with WMH volume (linear regression coefficient β=0.026, p=0.032) and PASAT (β=-0.0027, p=0.036). Periventricular normalized NAWM-ODI abnormality gradient correlated with WMH volume (β=-0.095, p<0.001), WMV (β=0.0024, p<0.001), GMV (β=0.0012, p<0.001), disease duration (β=-0.025, p=0.025), MMSE (β=0.033, p=0.004), SDMT (β=0.0037, p=0.045) and CVLT (β=0.0015, p=0.045). Periventricular normalized NAWM-ISOVF abnormality gradient correlated with WMH volume (β=0.075, p<0.001), WMV (β=-0.0022, p<0.001), GMV (β=-8.5×10^{-4}, p=0.003) and disease duration (β=0.033, p=0.005).

For AQP4+ NMOSD, periventricular normalized NAWM-NDI abnormality gradient correlated with GMV (β=-5.5×10^{-4}, p=0.022) and MMSE (β=0.016, p=0.035).

Periventricular normalized NAWM-ISOVF abnormality gradient correlated with GMV (β=-8.5 $\times$ 10^{-4}, p=0.002), number of relapses (β=0.054, p=0.008) and EDSS (β=0.066, p=0.029).

For AQP4- NMOSD, the periventricular normalized NAWM-NDI abnormality gradient correlated with WMV (β=-0.0014, p=0.016). Periventricular normalized NAWM-ODI abnormality gradient correlated with GMV (β=0.0011, p=0.020).

For MOGAD, periventricular normalized NAWM-ISOVF abnormality gradient correlated with WMV (β=-0.0023, p=0.012) and number of relapses (β=0.11, p=0.021).

For HCs, periventricular normalized NAWM-ISOVF gradient correlated with WMV (β=-0.0031, p<0.001) and GMV (β=-0.0017, p=0.032).

4. Discussion

The main findings of this study were: (1) compared to HCs, lower NDI within whole brain NAWM, and lower normalized NDI and ISOVF and higher normalized ODI in first periventricular ring were observed in MS, and lower NDI within whole brain NAWM were observed in AQP4+ NMOSD, while no significant NODDI metric abnormality was observed in AQP4- NMOSD or MOGAD; (2) periventricular normalized NAWM- NDI, ODI and ISOVF abnormality gradients were observed in MS, but not in AQP4+ NMOSD, AQP4- NMOSD or MOGAD; (3) different patterns of correlations of individual periventricular abnormality gradient of normalized NAWM NODDI metric with brain tissue volume (WMH volume, WMV and GMV) and clinical measures (disease duration, number of relapses, EDSS and cognitive score) were observed in MS, AQP4+ NMOSD, AQP4- NMOSD and MOGAD, independent of age, sex and whole brain NAWM NODDI metric.

NDI within whole brain NAWM and/or normalized NDI, ODI and ISOVF in first periventricular ring were abnormal in MS, and lower NDI within whole brain NAWM was found in AQP4+ NMOSD. MS has been demonstrated to have more prominent NAWM abnormalities, which may be attributed to chronic adaptive and innate immune responses, which could result in widespread axon degeneration (e.g., demyelination, axonal loss and fiber transection).(Absinta et al., 2021; Filippi et al., 2018) For NMOSD, the apparent discrepancy between AQP4+ and AQP4- patients may imply significant pathogenic differences between AQP4+ and AQP4- NMOSD, despite being clinically similar. AQP4 is crucial for maintaining the BBB permeability,(Papadopoulos and Verkman, 2012) and antibodies could cause widespread BBB leakage causing immune-mediated demyelination and axonal loss,(Bradl et al., 2018) which may explain why NAWM-NDI abnormalities in the AQP4+ NMOSD but not the AQP4- NMOSD. In MOGAD, MOG-antibodies are targeted against MOG in the oligodendrocyte, which plays a key role in myelination.(Takai et al., 2020) We found no significant abnormalities of diffusion measures in MOGAD NAWM, suggesting that they induce a more focal process than AQP4 antibodies. Moreover, previous reports also

demonstrated that cognitive decline and disability in MOGAD were more closely associated with GM abnormalities (e.g., cortical and subcortical atrophy) compared to subcortical WM.(Duan et al., 2021; Zhuo et al., 2020) However, as with the AQP4-NMOSD, these findings should be interpreted with caution due to a relatively small number (n=28) of MOGAD patients in this study.

Significant periventricular normalized NAWM- NDI, ODI and ISOVF abnormality gradients were observed in MS but not in AQP4+ NMOSD, AQP4- NMOSD or MOGAD at group-level. The findings in MS are consistent with previous studies.(Brown et al., 2017; Liu et al., 2015; Poirion et al., 2021) The absence of a periventricular NAWM abnormality gradient in AQP4+ NMOSD, AQP4- NMOSD and MOGAD suggests a distinct pathogenesis of NAWM abnormalities in these MS mimics.(Cacciaguerra et al., 2021) From the present results we cannot determine whether the gradient in NAWM periventricular NODDI metric abnormalities in MS is due to demyelination, axonal loss or another process. Several possible mechanisms for the gradient of NAWM abnormalities have been proposed, including CSF- or ependyma- mediated processes, venular leukocyte infiltration, hypoperfusion of brain parenchyma in watershed regions, or WM degeneration secondary to the periventricular lesions.(Brown et al., 2017; Poirion et al., 2021) Previous work has found that NDI is weighted more toward neurite density, while ODI is weighted towards fiber orientation, and ISOVF is associated with the free water fraction in WM tissues,(Kamiya et al., 2020; Zhang et al., 2012) but none of these measures are entirely specific and reduced neuro-axonal density, axonal transection, and demyelination could contribute to the observed microstructural abnormality simultaneously.(Gajamange et al., 2018; Lassmann, 2010) Periventricular WMH volume, WMH-NODDI metric gradients were observed in these demyelinating diseases and HCs, suggesting a common lesion spatial distribution pattern, which may result from a potential common CSF-mediated processes within WMH lesion.

In MS, consistent with previous PET and MTR findings,(Poirion et al., 2021) the

NAWM periventricular NODDI metric abnormality gradient was associated with both WM and GM atrophy, supporting the concept that they all reflect the same process (e.g. neuro-axonal loss), that they are pathogenically linked (e.g. periventricular axonal damage leading to tract-mediated GM atrophy), or are influenced by the same factor (e.g. mediated by CSF). Intriguingly, NAWM periventricular NODDI metric abnormality gradients were correlated with GMV in AQP4+ NMOSD but with WMV in AQP4- NMOSD, indicating distinct underlying interactions of WM and GM damage. For MOGAD, only significant correlation was observed for NAWM periventricular ISOVF abnormality gradient with WMV, indicating periventricular NAWM abnormality may be mild and independent of WMH volume and GM alterations. Additionally, the associations of periventricular NODDI metric abnormality gradient with WMV and GMV in HCs were similar to those in MS, indicating the potential CSF-mediated physiological or pathological process may present in normal neuro-degeneration,(Erickson and Banks, 2019; Porcher et al., 2021) which may be strengthened by inflammatory cytokine in MS.

The association of NAWM NODDI metric abnormality gradient with disease duration in MS, while with number of relapses in AQP4+ NMOSD and MOGAD supported the chronic inflammatory related disease course in MS, and relapse-related brain damages in AQP4+ NMOSD and MOGAD. We found associations between the NAWM NODDI metric abnormality gradient and cognitive impairment in MS, consistent with previous findings.(Brown et al., 2017; Liu et al., 2015) The NAWM periventricular ISOVF abnormality gradient was associated with EDSS in AQP4+ NMOSD, indicating the periventricular abnormalities within NAWM at individual-level may be partially account for physical disability. For MOGAD, no significant correlation was observed for NAWM periventricular NODDI metric abnormality gradient with cognitive decline or physical disability. Collectively, our findings indicate that microstructural abnormality gradients reflected by normalized NODDI metrics have distinct clinical significance in MS, NMOSD and MOGAD.

There are several limitations in the current study. First, the sample size of AQP4- NMOSD and MOGAD were relatively small and the age of patient with AQP4+ NMOSD is relative higher. However, the current findings still provide a further understanding of the distinct microstructural alterations in AQP4+ NMOSD, AQP4- NMOSD and MOGAD, which give a clue of the underlying different pathogenies and therapeutic targets. Studies with larger sample sizes especially for AQP4- NMOSD and MOGAD and age-matched disease and HC groups would help confirm the current findings. Second, this single-center study has a high image and clinical homogeneity, which could avoid the potential bias caused by different ethnicity, diagnostic criteria, treatment, scanner or MR acquisition protocol in multi-center studies, but external validations would help strengthen the significance of the current findings. Third, the disease duration was relatively short in each patient group, which may hamper to explore the long-term effect of the disease state on the diffusion gradient especially for NMOSD and MOGAD. However, the current findings still determined a diffusion gradient in early MS, indicating a different pathogenesis from its mimics. Longitudinal study would be performed to explore the long-term profile of the potential diffusion gradient especially in NMOSD and MOGAD. Last, the NODDI metrics were sensitive to microstructural alterations in both WM and GM, which was sufficient to investigate the diffusion gradient in WM but not so sensitive to the demyelination compared to MTR. Additionally, given the resolution of the NODDI data we could not look for an outside-in cortical gradient (as already observed in MS using high resolution MTR).(Brown et al., 2020; Rudko et al., 2016) Further study was warrant to investigate both the NAWM gradient and cortical gradient on diffusion and magnetic transfer imaging metrics.

5. Conclusion

Periventricular NAWM microstructural abnormality gradients were present in MS but absent in NMOSD or MOGAD, suggesting that in MS this is the result of a specific pathological process rather than a generic response to brain inflammation. We also found differences between AQP4+ and AQP4- NMOSD, raising the possibility that while they share common clinical characteristics, they are pathogenically distinct. Different correlations between individual periventricular NAWM microstructural abnormality gradients and clinical measures were identified between diseases, indicating different clinical significance of the NAWM diffusion gradients.

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Declaration of interest

J.S, SY.X, DC.T, YY.D, XL.X, S.L, GM.C, FD.S, ZZ.Z, XH.Z and Y.L declared there is no conflict of interest.

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