Comparison of brain and spinal cord magnetic resonance imaging features in neuromyelitis optica spectrum disorders patients with or without aquaporin-4 antibody

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ABSTRACT

Background: The spinal cord and brain measurements are rarely investigated in neuromyelitis optica (NMO) patients with and without antibodies to aquaporin-4 (AQP4), directly compared to multiple sclerosis (MS) patients.

Objectives: To investigate magnetic resonance imaging (MRI) features of both brain and spinal cord in NMO patients with and without antibodies to AQP4, compared with MS patients and healthy controls (HC).

Methods: We recruited 55 NMO including 30 AQP4 (+) and 25 AQP4 (–), 25 MS and 25 HC. Brain and spinal cord MRIs were obtained for each participant. Brain lesions (BL), whole brain and deep grey matter volumes (DGMV), white matter diffusion metrics and spinal cord lesions were measured and compared among groups.

Results: The incidence of BL was lower in the AQP4 (+) group than in the AQP4 (–) and MS groups ($p < 0.05$). In the AQP4 (+) group, there was a lower incidence of infratentorial lesions (ITL) and higher spinal cord lesions length than in the MS group ($p < 0.05$). The thalamic and hippocampal volumes were smaller in the AQP4 (–) group and MS group than in the HC group ($p < 0.05$).

Conclusions: The NMO patients with AQP4 (–) showed higher prevalence of BL, ITL, and similar spinal cord lesion length, compared to AQP4 (+), and demonstrated deep grey matter atrophy, suggesting an intermediate phenotype between that of typical MS and NMO.

1. Introduction

Neuromyelitis optica (NMO) is an autoimmune, inflammatory and demyelinating disease of the central nervous system (CNS). This syndrome is clinically characterized by recurrent attacks of optic neuritis and myelitis (Wingerchuk et al., 2007; Wingerchuk et al., 2006). The anti-aquaporin-4 (AQP4) antibody, which was first discovered in 2004, has been claimed to play a pathogenic role in NMO and can be used to achieve an early diagnosis (Lennon et al., 2004; Weinshenker et al., 2006). However, AQP4 antibody positivity is not universal in patients with clinically definite NMO, with reported

sensitivities ranging from approximately 30% to 90% (Cabrera-Gomez et al., 2009; Weinshenker et al., 2006).

In case series it has been demonstrated that lesions on brain magnetic resonance imaging (MRI) are common in NMO and showed differences between anti-AQP4 antibody positive and negative patients. A study in Cuba indicated that anti-AQP4 antibody positive NMO cases had more brain lesions than anti-AQP4 antibody negative NMO cases (Cabrera-Gomez et al., 2009). Whilst, Japanese and English investigators observed that brain lesions fulfilling the Barkhof criteria were significantly more common in patients without AQP4 antibodies (AQP4-Ab) than with autoimmune antibodies (Downer et al., 2012;

Abbreviation: ANOVA, analysis of variance; AQP4, aquaporin-4; BL, Brain lesions; CNS, central nervous system; DGM, deep grey matter; DGMV, deep grey matter volume; DTI, diffusion tensor images; EDSS, expanded disability status scale; FA, fractional anisotropy; GM, Grey matter; GMV, Grey matter volume; HC, healthy controls; ITL, infratentorial lesions; MD, mean diffusivity; MOG, myelin oligodendrocyte glycoprotein; MRI, magnetic resonance imaging; MS, Multiple sclerosis; NGMV, Normalized grey matter volume; NWBV, Normalized whole-brain volume; NWMV, Normalized white matter volume; NMO, Neuromyelitis optica; RRMS, relapsing-remitting MS; TBSS, tract-based spatial statistics; TE, echo time; TR, repetition time; WBV, whole brain volume; WMV, white matter volume; WM, white matter; LETM, Longitudinally extensive transverse myelitis

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Table 1
Demographic and clinical characteristics.

	AQP4 (+)	AQP4 (–)	MS	HC	P value
Gender (Male/ Female)	6/24	7/18	9/16	7/18	0.625 ¹
Age (year)	48.5 ± 11.6 (22–68)	46.4 ± 15.6 (17–66)	43.3 ± 9.6 (24–59)	45.9 ± 9.2 (25–63)	0.455 ²
Disease duration (year)	5.91 ± 5.04 (0–20)	4.01 ± 3.28 (0–13)	4.13 ± 5.81 (0.25–26)	–	0.312 ²
Number of attacks	3.7 ± 3.4 (1–17)	3.3 ± 2.0 (1–11)	2.8 ± 1.4 (2–7)	–	0.479 ²
EDSS	3.3 ± 2.0 (0–7)	3.4 ± 1.9 (0–8.5)	2.7 ± 1.6 (1–6)	–	0.427 ²

AQP4 (+)=NMO patients seropositive for anti-aquaporin-4 antibodies; **AQP4 (–)**=NMO patients seronegative for anti-aquaporin-4 antibodies; **MS**=multiple sclerosis patients; **HC**=Healthy controls; **EDSS**=expanded disability status scale. P-value: groups were compared using Chi-Square test¹ and ANOVA test². Data are presented as Mean ± SD (range).

Matsushita et al., 2010). GM atrophy and WM diffusion abnormalities in NMO patients were reported by recent studies (Chanson et al., 2013; Duan et al., 2012; Kimura et al., 2014; Liu et al., 2012; Pichiechio et al., 2012; Rueda Lopes et al., 2012; Zhang et al., 2014), but whether these changes differ between antibody-positive and antibody-negative NMO patients is unknown. Some studies have investigated the different patterns of spinal cord lesion between anti-AQP4 antibody positive and negative patients (Jarius et al., 2012), showing that the total spinal cord lesion load was higher, as well as lesions ≥6 vertebral segments, entire spinal cord involvement and lesions with Gd-enhancement (Liu et al., 2013), were more common in seropositive patients. As far as we know, there has not yet been a study investigating MRI features of both the brain and spinal cord in NMO patients (AQP4 (+) vs. AQP4 (–)), compared with well-matched MS patients and HC. Recent studies have identified antibodies against myelin oligodendrocyte glycoprotein (MOG-Abs) in NMO spectrum disorders (NMOSD) (Kitley et al., 2012, 2014; Ramanathan et al., 2016; Willison and Linington, 2012). The similarities and differences of MRI characteristics between MOG (+) and (–) patients also remain unknown.

In this study, we assessed brain and spinal cord MRI characteristics including brain and spinal cord lesions, brain GM atrophy, WM diffusion measurements between AQP4-Ab-positive and AQP4-Ab-negative clinically definite NMO patients, directly compared with age and gender matched MS and healthy controls. Additionally, we compared MRI characteristics of MOG (+) and (–) patients in AQP4 (–) patients.

2. Materials and methods

2.1. Subjects

The study was approved by the local ethics committee and written informed consent was obtained from all patients and volunteers. We recruited 69 NMO patients (14 men and 55 women) from the

Department of Neurology in Tianjin Medical University General Hospital. The NMO diagnosis was determined according to the 2006 diagnostic criteria (Wingerchuk et al., 2006). All patients had optic neuritis and myelitis, and met 2 of the 3 supporting criteria including AQP4 antibody positivity, negative brain MRI or not meeting the diagnostic criteria for MS, and a spinal cord lesion involving at least three vertebral segments. For direct comparison with NMO patients, we selected 28 relapsing-remitting MS (RRMS) patients (9 men, 19 women) fulfilling the McDonald criteria (Polman et al., 2005) by matching them for age, gender, disease duration and EDSS score to the NMO group from an ongoing MS cohort study. 14 NMO and 3 MS cases were excluded due to incomplete clinical assessment (n=3; 2 NMO, 1 MS); MRI image artifacts (n=9; 7 NMO, 2 MS) or the presence of other CNS diseases (n=5; 5 NMO, 0 MS). 55 NMO patients and 25 MS cases were entered into the final analysis. 32 Of 55 NMO patients were relapsing NMO, 30 NMO patients were AQP4-seropositive (AQP4-Ab (+)) and 25 were seronegative (AQP4-Ab (–)). 4 patients were MOG-seropositive in the NMO AQP4-Ab (–) patients, and one patient was double positive for anti-AQP4 and MOG antibodies. All MS patients were relapsing-remitting patients and AQP4-seronegative. The main demographic and clinical characteristics, including age, disease duration, age at disease onset and expanded disability status scale (EDSS) of the patients studied are reported in Table 1. Additionally, we chose twenty-five age and gender matched healthy controls (HCs) (7 men, 18 women) with no previous history of neurological dysfunction and with normal findings on neurological examination and MRI (Table 1).

2.2. AQP4 and MOG antibody assay

Enhanced green fluorescent protein tagged AQP4/EGFP and MOG/EGFP fusion protein transfected human embryonic kidney cells (HEK-293) were produced as previously described (Yang et al., 2014; Zhang et al., 2015). Assays for anti-AQP4 and anti-MOG antibodies were carried out at least twice for each sample, and those that gave a positive or negative result twice were deemed to be positive or negative.

2.3. Image acquisition

MR imaging was performed on a 3.0 T MR system using an eight-channel phased array head coil in Tianjin Medical University General Hospital. Axial T2-weighted turbo spin echo (40 interleaved slices, repetition time (TR)=6816 ms, echo time (TE)=91 ms, echo train length=32, section thickness=3 mm, in-plane resolution 1 mm²) was used for lesion detection. High-resolution anatomical images were acquired using sagittal 3D T1-weighted images sequence (number of slices=191; TR=8.16 ms; TE=3.18 ms; flip angle (FA)=12°; slice thickness=1 mm; in-plane resolution 1 mm²), which was used for brain volume measurement. Furthermore we acquired 2D echo-planar diffusion tensor images (DTI) [number of slices=69; TR=7100 ms, TE=61.3 ms, slice thickness=2 mm, in-plane resolution 2 mm²; 50 non-collinear diffusion gradients (b=1000 s/mm²) and three non-diffusion-weighted images (b=0 s/mm²)] for measuring WM tract integrity. All the spinal cord imaging was acquired on the same scanner

Table 2
Clinical characteristics of 5 patients with MOG-Abs in NMO (–) patients.

Patients	Age (year)	Sex	No. of attacks	Disease duration (year)	EDSS	Diagnosis	AQP4 Abs	MOG Abs	Other serum Abs	Treatment
1	22	M	2	6	2	NMO	negative	positive	negative	CS
2	50	M	2	1	1	NMO	negative	positive	negative	None
3	52	M	6	5	6	NMO	negative	positive	negative	CS
4	66	F	2	8	6	NMO	negative	positive	negative	CS

AQP4=Anti-aquaporin-4; **Abs**=antibodies; **CS**=oral corticosteroids; **EDSS**=expanded disability status scale; **F**=female; **M**=male; **MOG**=myelin oligodendrocyte glycoprotein; **No. of attacks**=number of attacks.

Fig. 1. **A** shows significant differences in the incidence of brain lesions (BL) ($P=0.001$) and infratentorial lesions (ITL) ($P=0.001$) between the three groups [AQP4 (+), AQP4 (–) and MS]. **B** shows no difference in brain lesion volume between the three groups ($P=0.070$). **C** shows no difference in the incidence of BL ($P=1.000$) and ITL ($P=0.422$) between the MOG (+) and MOG (–) groups. **D** shows no difference in brain lesion volume between the MOG (+) and MOG (–) groups ($P=0.970$). STL=supratentorial lesion; ITL=infratentorial lesion; NBL=no brain lesion.

Fig. 2. Axial T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI from MS patients demonstrates lesions involving juxtacortical (A1 arrow), periventricular (A2 and A3 arrow), and infratentorial (A4 arrow) regions. Axial T2 FLAIR MRI show some subcortical punctate lesions, most of them < 3 mm (B1 arrow), extensive subcortical white matter lesions (B2 arrow), periaqueductal lesions (B3 arrow) from AQP4 (–) patients, and dorsal brainstem lesions adjacent to the fourth ventricle was depicted using T2-weighted MRI (B4 arrow). Only some subcortical and deep white matter punctate and patchy lesions were observed using T2 FLAIR MRI (C1–C3 arrow), and corpus callosum lesions in T2 MRI (C4 arrow) from AQP4 (+) cases.

Fig. 3. **A** shows comparison of the number of spinal cord lesion between the three groups ($P=0.484$). **B** reveals a significant difference in the total length of spinal cord lesions between the three groups ($P=0.001$). **C** shows no difference in the number of spinal cord lesions between the MOG (+) and MOG (–) groups ($P=0.599$). **D** shows no difference in the total length of spinal cord lesions between the MOG (+) and MOG (–) groups ($P=0.719$).

(sagittal T1-weighted SE, TR=530 ms; TE=12 ms; slice thickness=3 mm; sagittal T2-weighted TSE, TR=2300 ms; TE=90 ms; slice thickness=3 mm; Axial T1-weighted SE, TR=530 ms; TE=13 ms; slice thickness=4 mm; Axial T2-weighted TSE, TR=4300 ms; TE=120 ms; slice thickness=4 mm).

2.4. Image analysis

Marking and measurement of hyperintense brain lesions (T2LV) on T2-weighted images was performed by an experienced neurologist using MRIcro software. Infratentorial lesions were defined as lesions in the cerebellum, pons, medulla or mesencephalon. Normalized whole-brain volume (NWBV), grey matter volume (NGMV), and white matter volume (NWMV) were computed using the 3D T1-weighted images and SIENAX (part of FSL 5.0.4) (Smith et al., 2002). In order to avoid classification of lesions as grey matter, T2 lesion masks were co-registered to the 3D T1-weighted images to perform lesion filling, using individually averaged normal appearing white matter values prior to brain tissue volume segmentation. Deep grey matter (DGM) volumes including the thalamus and hippocampus were quantified by the FIRST (also part of FSL) (Patenaude et al., 2011). The left and right volumes for each DGM structure were averaged and normalized by multiplying with the V-scaling from SIENAX. The diffusion metrics (fractional anisotropy, FA, and mean and axial diffusivity) were quantified using the diffusion-weighted images and tract-based spatial statistics (TBSS; also part of FSL). The mean FA image was thresholded at 0.2 and skeletonized. Each subject's FA and MD values were projected onto the mean FA skeleton. The average FA and MD values of the whole skeleton were calculated for each subject.

2.5. Statistical analysis

Analyses were performed by using SPSS software (Version 18; SPSS, Chicago, Ill). Kolmogorov-Smirnov tests were used together with visual inspection of histograms to assess normality of the variables. To compare gender and the incidence of brain lesions and infratentorial lesions between the AQP4 (+) group, AQP4 (–) group, MS group and HC group, we used the Chi-Square test. Comparisons of the demographic and MRI parameters between AQP4 (+) patients, AQP4 (–) patients, MS patients and HCs were conducted using analysis of variance (ANOVA) and the Tukey honestly significant difference test was used for post hoc comparisons. Comparisons of the MRI parameters between MOG (+) group and MOG (–) group, were performed using the *t*-test or Mann-Whitney *U* test according to the normality of the variables. *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Demographics and clinical characteristics

As shown in Table 1, no significant differences were observed in age and gender among the four groups (NMO AQP4-antibody positive, NMO AQP4 antibody negative, MS and HC). There were no significant differences in EDSS and disease duration between the AQP4 (+), AQP4 (–) and MS patients. The clinical characteristics of the 4 NMO patients with MOG antibodies are shown in Table 2.

3.2. Brain lesions

The incidence of brain lesions (BL) and infratentorial lesions (ITL) showed significant differences between the three groups ($p=0.001$ and $p=0.001$, respectively), in which the AQP4 (–) ($p=0.032$) and MS

Fig. 4. Sagittal T2-weighted MRI of the cervical spinal cord demonstrates a short (< 3 vertebral segments), asymmetric, non-transverse myelitis (A1-A3 arrows) in an MS patient. Sagittal T2-weighted MRI shows a typical longitudinally extensive transverse myelitis (LETM) lesion (B1 and B3 arrows) and segmental spinal cord atrophy (B2 arrows) in an AQP4 (-) patient. Sagittal T2-weighted MRI shows an LETM lesion involving all of the cervical spinal cord (C1 arrows) in an AQP4 (+) patient. Cervical LETM extending into the medulla and longitudinally extensive segments of spinal cord atrophy as shown by T2-weighted MRI using sagittal and axial (C2 and C3 arrows) in an AQP4 (+) patient.

groups ($p=0.000$) showed a higher prevalence of BL than the AQP4 (+) group, no significant difference between the AQP4 (-) group and MS group ($p=0.247$) was observed (Fig. 1A). There was a higher incidence of ITL in the MS group than in AQP4 (+) group ($p=0.001$), but no difference was identified in NMO patients with or without AQP4-Ab ($p=0.088$) (Fig. 1A). None of the AQP4 (+) patients presented with ITL. There was no significant difference in the brain lesion volumes between the three groups ($p=0.070$) (Fig. 1B). Examples of the brain MRI images of the three groups are shown in Fig. 2. Moreover, the incidence of brain lesions (BL), infratentorial lesions (ITL) and brain lesion volume showed no differences between the MOG (+) and MOG (-) groups of the AQP4 (-) patients ($p=1.000$, $p=0.422$, $p=0.970$, respectively) (Fig. 1C and D).

3.3. Spinal cord lesions

The number of spinal cord lesions was not significantly different among the three patient groups ($p=0.484$) (Fig. 3A). The total length of spinal cord lesions was higher in the AQP4 (+) than the MS group ($p=0.000$). No significant difference was observed between the AQP4 (-) and MS group ($p=0.069$) or the AQP4 (+) and AQP4 (-) group ($p=0.193$) (Fig. 3B). In the AQP4 (-) group, there were no significant differences in the number of spinal cord lesions and total length of spinal cord lesions between the MOG (+) and MOG (-) groups ($p=0.599$ and $p=0.719$ respectively) (Fig. 3C and D). Examples of the spinal cord MRI images of the three groups are shown in Fig. 4.

3.4. Brain volume

Compared with the HCs, whole brain volume (WBV) and white matter volume (WMV) were decreased in the MS group ($p=0.009$; $p=0.001$, respectively) (Fig. 5A and B). The MS patients showed reduction of white matter volume (WMV) compared to the AQP4 (+) patients ($p=0.005$). Grey matter volume (GMV) showed no statistical differences among the four groups ($p=0.072$) (Fig. 5C). In AQP4 (-) patients, WBV, GMV and WMV were similar between patients with and without MOG antibodies ($p=0.266$; $p=0.120$; $p=0.941$ respectively) (Fig. 5D–F). Thalamic and hippocampal volumes, belonging to deep grey matter volume (DGMV), were obviously different among the four groups ($p=0.039$; $p=0.029$). Thalamic and hippocampal volumes were decreased in the AQP4 (-) group ($p=0.023$; $p=0.021$) and the MS group ($p=0.006$; $p=0.021$) compared to the HC group (Fig. 6A and B). However, no differences were found in the thalamic or hippocampal volumes of AQP4 (-) patients with or without MOG antibodies ($p=0.505$; $p=0.941$) (Fig. 6C and D).

3.5. White matter tract integrity

We used two DTI measurements, (whole brain FA and MD), to evaluate the integrity of brain white matter. There were no significant differences in either FA or MD values among three patient groups (AQP4 (+), AQP4 (-) and MS) ($p=0.452$) (Fig. 7A) ($p=0.018$) (Fig. 7B). The only significant difference was observed between the MD of MS patients and HCs ($p=0.004$) (Fig. 7B). There were no significant differences between the MOG (+) and MOG (-) groups of the AQP4

Fig. 5. A shows the whole brain volume (WBV) in the MS group is smaller than in the HC group ($P=0.009$). However, there is no difference between the AQP4 (-) group, AQP4 (+) group and MS group. B shows the white matter volume (WMV) in the MS group is smaller than in the HC group ($P=0.001$) and the AQP4 (+) group ($P=0.027$), but there is no difference between the AQP4 (-) group and the AQP4 (+) group ($P=0.699$). C shows the grey matter volume (GMV) are not different between the four groups ($P=0.072$). D, E, F show no difference between the MOG (+) group and the MOG (-) group in WBV ($P=0.266$), WMV ($P=0.941$) and GMV ($P=0.120$).

(-) patients in FA ($p=0.089$) and MD ($p=0.212$) showed (Fig. 7C and D).

4. Discussion

In this study, we investigated the brain and spinal cord MRI characteristics in NMO patients, and searched for differences between AQP4-seropositive and -seronegative NMO patients. Furthermore we compared the MOG (+) and MOG (-) patients of the AQP4 (-) NMO group. We demonstrated that (1) the incidence of brain lesions and infratentorial lesions were significantly higher in the AQP4 (-) patients than in the AQP4 (+) patients. The prevalence of brain lesions in seronegative NMO cases was similar to MS cases. (2) Total spinal cord lesion length was higher in AQP4 (+) patients compared to MS patients, but no significant differences were identified between AQP4 (+) and (-) or between AQP4 (-) and MS. (3) Deep grey matter atrophy including in the thalamus and hippocampus was predominantly found in AQP4 (-) patients. Whole brain atrophy was subtle in both AQP4 (+) and (-) patients. (4) Whole brain white matter diffusion was not significantly altered in both AQP4 (+) and (-) patients. (5) There were no significant differences of MRI characteristics including lesions, brain volume and white matter integrity between MOG (+) and MOG (-) patients.

Previous studies have reported that brain abnormalities are seen in the majority of NMO cases. Brain lesions are present in about half of patients at presentation and increase with disease progression (Cabrera-Gomez and Kister, 2012; Kiyat-Atamer et al., 2013; Pittock et al., 2006). The current study demonstrated that the incidence of brain lesions in NMO cases is 60%, of which the incidence was 46.7% in seropositive NMO cases and 76% in seronegative NMO cases. MS patients demonstrated even higher brain lesion prevalence (92%). This finding is consistent with previous studies (Asgari et al., 2013; Jarius et al., 2012). Infratentorial (including the cerebellum and brain stem) lesions have seldom been compared between NMO and MS. Our study has shown that the incidence of infratentorial lesions in AQP4 (-) patients is similar to MS cases. Interestingly, none of the AQP4 (+) cases in our cohort have infratentorial lesions.

Longitudinally extensive transverse myelitis (LETM) is an MRI feature of NMO, which is predominantly central in position and involves grey as well as white matter (Downer et al., 2012; Nakamura et al., 2008; Zhong et al., 2012). In an attempt to shed light on the difference of spinal cord lesions between AQP4 (+) and AQP4 (-) cases, we examined the number of spinal cord lesions and the total extent of spinal cord lesions in each patient. We found that there were no significant differences in the number of spinal cord lesion between the three groups. The AQP4 (+) group had longer spinal cord

Fig. 6. **A** shows thalamic volume in the MS group ($P=0.006$) and the AQP4 (–) group ($P=0.023$) are smaller than in the HC group. **B** shows hippocampal volume in the MS group ($P=0.021$) and the AQP4 (–) group ($P=0.021$) are smaller than in the HC group. **C**, **D** shows no difference in the volumes of thalamus ($P=0.505$) and hippocampus ($P=0.941$) between the MOG (+) group and the MOG (–) group.

lesions than the MS group, but a similar total lesion length compared with the AQP4 (–) group. The length of spinal cord lesions was higher in the AQP4 (+) group than in the MS group. The difference in brain lesions, especially ITL lesions, but not in spinal cord lesions between AQP4 (+) and (–) patients implies that brain lesions are key features differentiating AQP4 (+) vs (–).

As far as we know, progressive development of brain atrophy is a well-known feature in MS (Duan et al., 2012; Schwarz et al., 2001; Liu et al., 2015), our finding agrees with the results that the volumes of the whole brain, grey matter and white matter are reduced in MS patients compared to HC. In AQP4-seronegative NMO patients, only some deep grey matter atrophy was identified, including the thalamus and hippocampus, in the present study, suggesting that AQP4 (–) NMO patients share some common pattern of pathological mechanism causing brain damage.

In this study, we evaluated the global white matter changes of patients by measuring FA and MD across the whole brain using TBSS. Rueda Lopes et al. (2012) and Liu et al. (2012) have reported that there is extensive white matter damage in patients with NMO. However, we did not find changes in WM diffusion in NMO patients in our study, which may be due to the fact that we only measured DTI parameters of the whole brain, while previous studies confined their measurements to specific white matter tracts such as optic radiation or corticospinal tract. These findings indicate that white matter injury in NMO is subtle, and no differences in diffusion metrics were identified between AQP4 (+) and (–).

Experimental studies have increasingly indicate that MOG antibodies can be found in patients with NMO (Kitley et al., 2014; Mader et al., 2011; Probstel et al., 2015; Willison and Linington, 2012). Previous studies imply that MOG serostatus is another important biomarker for NMO (Kitley et al., 2014; Willison and Linington, 2012). In order to demonstrate the clinical and imaging characteristics

of NMO, we tested MOG antibodies in all patients and healthy individuals. In our study, most MOG-seropositive cases come from patients with an AQP4-seronegative NMO phenotype. And our data confirmed several studies demonstrating MOG-seropositive patients tend to be males (Kitley et al., 2014; Probstel et al., 2015). Our study did not find any differences between MOG-seropositive and MOG-seronegative patients in terms of MRI characteristics.

Our study has several limitations. First, this is a cross-sectional study using structural MRI measurements and the sample size is relatively small. The current results warrant further validation by large sample studies. Second, our research is mainly focused on whole brain imaging changes of patients, rather than concentrating on specific regions of interest or white matter tracts. Finally, this study only investigated the structural MRI measurement in patients. Future studies with fMRI techniques are needed to extend our findings by combining functional and structural measures.

5. Conclusion

The NMO patients without AQP4 antibodies showed a higher prevalence of brain lesions, infratentorial lesions, and similar spinal cord lesion length, compared to AQP4 (+) patients, and demonstrated deep grey matter atrophy without significant whole brain atrophy and diffusion alterations compared to HCs, suggesting an intermediate phenotype between typical MS and NMO. No significant differences of MRI characteristics were identified between patients with different MOG serostatus in AQP4 (–) NMO patients.

Conflict of interest/role of funding source

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Fig. 7. **A** shows no difference in FA among the four groups ($P=0.452$). **B** shows MD in the MS group is higher than in the HC group ($P=0.004$), but there is no difference between the AQP4 (–) group, AQP4 (+) group and MS group. **C** and **D** shows no difference in indicators of the integrity of white matter tracts between the MOG (+) group and the MOG (–) group ($P=0.089$) ($P=0.212$).

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