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Evidence of subclinical quantitative retinal layer abnormalities in AQP4-IgG seropositive NMOSD

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Abstract

Background: Prior studies have suggested that subclinical retinal abnormalities may be present in aquaporin-4 (AQP4)-IgG seropositive neuromyelitis optica spectrum disorder (NMOSD), in the absence of a clinical history of optic neuritis (ON).

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Disclosures

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Elias Sotirchos has received speaker honoraria from Viela Bio and has served on scientific advisory boards for Viela Bio and Genentech.

Conflict of Interest

The authors report no conflict of interest.

Objective: To compare retinal layer thicknesses at the fovea and surrounding macula between AQP4-IgG+ NMOSD eyes without a history of ON (AQP4-nonON) and healthy controls (HC).

Methods: In this single-center cross-sectional study, 83 AQP4-nonON and 154 HC eyes were studied with spectral-domain optical coherence tomography (OCT).

Results: Total foveal thickness did not differ between AQP4-nonON and HC eyes. AQP4-nonON eyes exhibited lower outer nuclear layer (ONL) and inner photoreceptor segment (IS) thickness at the fovea (ONL: $-4.01 \pm 2.03 \mu\text{m}$, $p=0.049$; IS: $-0.32 \pm 0.14 \mu\text{m}$, $p=0.029$) and surrounding macula (ONL: $-1.98 \pm 0.95 \mu\text{m}$, $p=0.037$; IS: $-0.16 \pm 0.07 \mu\text{m}$, $p=0.023$), compared to HC. Macular retinal nerve fiber layer (RNFL: $-1.34 \pm 0.51 \mu\text{m}$, $p=0.009$) and ganglion cell + inner plexiform layer (GCIPL: $-2.44 \pm 0.93 \mu\text{m}$, $p=0.009$) thicknesses were also lower in AQP4-nonON compared to HC eyes. Results were similar in sensitivity analyses restricted to AQP4-IgG+ patients who had never experienced ON in either eye.

Conclusions: AQP4-nonON eyes exhibit evidence of subclinical retinal ganglion cell neuronal and axonal loss, as well as structural evidence of photoreceptor layer involvement. These findings support that subclinical anterior visual pathway involvement may occur in AQP4-IgG+ NMOSD.

Keywords

neuromyelitis optica; retina; fovea; aquaporin-4; optical coherence tomography

Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is an immune-mediated inflammatory disease of the central nervous system, characterized by severe attacks of optic neuritis (ON), longitudinally extensive transverse myelitis, and, less frequently, brainstem and cerebral presentations.¹ The majority of cases of NMOSD are associated with aquaporin-4 (AQP4)-IgG seropositivity, which is considered to be pathogenic.² AQP4 is a water channel densely expressed on astrocytic end foot processes; binding of AQP4-IgG to astrocytic AQP4 results in activation of an inflammatory cascade including complement activation and recruitment of inflammatory cells, leading to astrocytic damage, demyelination, and neuro-axonal loss.³

AQP4 is expressed by two distinct cell types in the retina, including astrocytes in the retinal nerve fiber layer (RNFL) and, more abundantly, by Müller cells, which are a unique type of glial cell, the cell bodies of which are located in the inner nuclear layer (INL), with cell processes spanning from the inner to outer limiting membrane.^{4,5} Müller cells are five times more abundant in the macula, where they are concentrically distributed around the fovea, and fulfill many important roles in the retina, including maintenance of the homeostasis of the retinal extracellular compartment, release of neurotrophic factors, and degradation of glutamate.^{5–8} Gliosis of Müller cells leads to neuronal damage through disruption of these neuroprotective functions.⁶

Prior studies utilizing retinal optical coherence tomography (OCT) have reported lower thickness of the overall fovea in AQP4-IgG+ NMOSD eyes without a history of ON, as well as altered foveal morphology.^{9–11} Additionally, a recent study reported electrophysiologic evidence of Müller cell dysfunction and lower foveal outer nuclear layer (ONL) and inner

photoreceptor segment (IS) thickness in AQP4-IgG+ NMOSD eyes. Collectively, these findings support that subclinical retinal involvement targeting Müller glial cells may occur in NMOSD.⁵

In the current study, our objective was to assess for the presence of subclinical retinal abnormalities in AQP4-IgG+ NMOSD, by comparing retinal layer thicknesses at the fovea and the surrounding macula between AQP4-IgG+ NMOSD eyes without a history of ON (AQP4-nonON) and healthy controls (HC).

Materials and Methods

Standard protocol approvals, registrations, and patient consents

Johns Hopkins University Institutional Review Board approval was obtained for the study protocol, and written informed consent was obtained from all participants prior to study enrolment.

Study participants, clinical data and AQP4-IgG testing

Patients with AQP4-IgG seropositive NMOSD without a clinical history of ON or with previous unilateral ON were recruited from the Johns Hopkins Neuromyelitis Optica, Transverse Myelitis and Multiple Sclerosis (MS) Clinics between 2008 and 2020. All patients exhibited typical clinical disease courses and fulfilled the 2015 International Panel for Neuromyelitis Optica Diagnosis criteria for AQP4-IgG seropositive NMOSD (retroactively applied to patients recruited prior to 2015).¹ AQP4-IgG antibody testing was performed by commercially available assays (cell-based assay [CBA] or enzyme-linked immunosorbent assay [ELISA]). HC were recruited among patients' partners/spouses and Johns Hopkins staff.

Subjects with history of ocular surgery/trauma, glaucoma, diabetes mellitus, uncontrolled hypertension, or other ophthalmologic or neurologic disorders were excluded from the study.

OCT

Retinal imaging was performed with spectral-domain OCT (Cirrus HD-OCT, Model 5000; Carl Zeiss Meditec, Dublin, CA), as previously described.¹² Briefly, peripapillary data were obtained with the Optic Disc Cube 200×200 and Macular Cube scans were obtained using the 512×128 protocol. The scans were assessed in accordance with the OSCAR-IB criteria; scans with signal strength less than 7/10, or with artifact, were excluded from the study.^{13,14}

Peri-papillary retinal nerve fiber layer (pRNFL) thicknesses were estimated using the software incorporated in the Cirrus HD-OCT device. Segmentation of macular cube scans was performed by utilizing a previously described automated segmentation algorithm.¹⁵ Retinal layer thicknesses in the macular scans were assessed in the following primary regions of interest, centered at the fovea centralis: 1) an annulus with an internal diameter of 1 mm and an external diameter of 5 mm (macula excluding fovea) 2) a circle with 1 mm diameter (fovea). Additionally, we assessed retinal layer thicknesses in a circle with 2 mm diameter (fovea and parafovea), since this area was examined in a prior OCT study reporting specific retinal layer thinning at the fovea in AQP4-IgG+ NMOSD.⁵ All

acquired macular cube scans were segmented and qualitatively assessed in a blinded manner for segmentation accuracy and abnormalities of the retina or vitreoretinal interface, by two reviewers (AGF, ESV). The scans underwent rigorous quality control to confirm the accuracy of the segmentation, as well as to assess the presence of macular pathology (including microcystoid macular pathology, also referred in the literature as microcystic macular edema).¹⁶ Only scans passing the quality control process were included in the analyses. The following macular OCT measures were quantified: RNFL, ganglion cell+inner plexiform layer (GCIPL), INL, outer plexiform layer (OPL), ONL, IS, outer photoreceptor segment (OS), total retinal thickness. The photoreceptor complex (photoreceptor cell bodies [located in the ONL] and inner and outer photoreceptor segments) was also examined as a whole (ONL and photoreceptor segments; ONLPS).

OCT methods and results are reported in accordance with the consensus Advised Protocol for OCT Study Terminology and Elements (APOSTEL) recommendations.¹⁷

Visual Function

Monocular visual acuity (VA) was assessed using standardized retro-illuminated eye charts (Precision Vision, La Salle, IL) with habitual correction. High-contrast (100%) letter acuity (HCLA) was assessed with Early Treatment Diabetic Retinopathy Study charts (at 4m), and low-contrast (2.5% and 1.25%) letter acuity (LCLA) with Sloan Letter charts (at 2m). The maximum score for each chart is 70 letters, corresponding to a Snellen visual acuity of 20/10.

Statistical Methods

OCT and VA measures were compared between AQP4-nonON and HC eyes using linear generalized estimating equation (GEE) models, accounting for within-subject inter-eye correlations. Analyses examining whether disease duration is associated with OCT measures were also performed with GEE. All analyses were adjusted for age (as a continuous variable), sex, and race (as a dichotomous variable: African-American vs non-African-American).

To ensure that our results were not affected by chiasmal/optic tract involvement in patients with contralateral ON, we performed sensitivity analyses that were restricted to participants who had never experienced ON in either eye (AQP4-neverON).

Statistical analyses were performed using Stata 16 (StataCorp, College Station, TX). Analyses were based on a priori established research hypotheses, and consequently, adjustment for multiple comparisons was not performed. Statistical significance was defined as $p < 0.05$.

Results

A total of 51 AQP4-IgG+ patients (83 eyes) and 77 HC (154 eyes) were eligible for inclusion in the study (Table 1, Suppl. Figure 1). The AQP4 and HC cohorts were similar in terms of age, sex and race. In regard to clinical characteristics, 33 patients had never experienced a clinical episode of ON in either eye, while 18 patients had experienced

unilateral ON; in these cases, as per our inclusion criteria, only the non-ON eye was included in the analyses. Microcystoid macular pathology was not found in any of the AQP4-nonON or HC eyes. For a subset of AQP4-nonON (n=70 eyes) and HC (n=116 eyes), VA assessments were also available.

OCT measures at the macula excluding the fovea

Total retinal thickness of the macula excluding the fovea was lower in AQP4-nonON compared to HC eyes ($-6.25\mu\text{m}$; 95% CI: -10.85 to -1.65 ; $p=0.008$; Table 2; Figure 1a). This was driven by lower RNFL ($-1.34\mu\text{m}$; 95% CI: -2.35 to -0.33 ; $p=0.009$; Figure 1b), GCIPL ($-2.44\mu\text{m}$; 95% CI: -4.26 to -0.62 ; $p=0.009$; Figure 1c), ONL ($-1.98\mu\text{m}$; 95% CI: -3.85 to -0.12 ; $p=0.037$; Figure 1d) and IS ($-0.16\mu\text{m}$; 95% CI: -0.30 to -0.02 ; $p=0.023$; Figure 1e) thicknesses in AQP4-nonON eyes. ONLPS thickness was also found to be lower in AQP4-nonON eyes ($-2.04\mu\text{m}$; 95% CI: -4.07 to -0.01 ; $p=0.048$; Figure 1f). INL, OPL, and OS thicknesses did not differ between AQP4-nonON eyes and HC eyes.

OCT measures at the fovea

Retinal layer thicknesses at the fovea are summarized and compared between groups in Table 3. Total foveal thickness did not differ between AQP4-nonON and HC eyes (Figure 2). However, AQP4-nonON eyes had lower ONL ($-4.01\mu\text{m}$; 95% CI: -8.00 to -0.02 ; $p=0.049$; Figure 3a), IS ($-0.32\mu\text{m}$; 95% CI: -0.60 to -0.03 ; $p=0.029$; Figure 3b) and ONLPS ($-4.57\mu\text{m}$; 95% CI: -8.65 to -0.49 ; $p=0.028$; Figure 3c) thicknesses compared to HC eyes. Other retinal layer thicknesses did not differ between groups at the fovea. Findings were overall similar when examining a 2mm area (fovea and parafovea), and are shown in Suppl. Table 1.

OCT measures of the optic disc

Comparison of pRNFL thickness between AQP4-nonON and HC eyes revealed that pRNFL thickness was lower in AQP4-nonON eyes ($-2.79\mu\text{m}$; 95% CI: -5.69 to 0.11 ; $p=0.059$; Table 2, Figure 4); consistent with our findings for the macular RNFL, however this difference did not attain statistical significance.

Sensitivity analyses

In order to exclude effects due to chiasmal or optic tract involvement in patients with a prior history of contralateral ON, we performed sensitivity analyses limited to AQP4-IgG+ patients who had never experienced a clinical episode of ON in either eye (AQP4-neverON). The results for the OCT measures of the macula excluding the fovea were similar, with lower RNFL ($-1.33\mu\text{m}$; 95% CI: -2.51 to -0.16 ; $p=0.026$; Suppl. Figure 2b), GCIPL ($-2.59\mu\text{m}$; -4.80 to -0.39 ; $p=0.021$; Suppl. Figure 2c), and IS ($-0.16\mu\text{m}$; 95% CI: -0.31 to -0.02 ; $p=0.031$; Suppl. Figure 2e) thicknesses found in AQP4-neverON vs HC eyes (Suppl. Table 2). The comparisons for ONL ($-2.01\mu\text{m}$; 95% CI: -4.05 to 0.02 ; $p=0.052$; Suppl. Figure 2d) and ONLPS ($-2.04\mu\text{m}$; 95% CI: -4.22 to 0.14 ; $p=0.07$; Suppl. Figure 2f) were of similar magnitude, although they did not remain statistically significant, likely due to the smaller sample size. For foveal retinal layer thicknesses, the magnitude of the estimated differences was similar for the ONL ($-3.78\mu\text{m}$; 95% CI: -8.24 to 0.69 ; $p=0.10$),

IS ($-0.28\mu\text{m}$; 95% CI: -0.64 to 0.09 ; $p=0.14$) and ONLPS ($-3.99\mu\text{m}$; 95% CI: -8.56 to 0.58 ; $p=0.09$), but these differences did not remain statistically significant (Suppl. Table 3, Suppl. Figures 3 and 4). Results were similar when examining the fovea plus parafoveal region (2mm diameter; Suppl. Table 4). Finally, findings for the pRNFL thickness remained consistent with the primary analysis ($-3.20\mu\text{m}$; 95% CI: -6.72 to 0.32 , $p=0.075$; Suppl. Table 2, Suppl. Figure 5).

Association of demographic characteristics with OCT measures

In the aforementioned analyses, we observed marked differences in retinal thicknesses by sex and race, in line with prior studies.^{18–21} The sex- and race-related differences were of similar magnitude in AQP4-nonON and HC eyes (data not shown); the coefficients for male sex and African-American race from our models (adjusted differences across AQP4-IgG+ NMOSD and HC eyes) are shown in Suppl. Tables 5 and 6. Notably, African-American race was associated with markedly lower foveal thickness ($-18.65\mu\text{m}$; 95% CI: -26.00 to -11.29 ; $p<0.001$), while male sex was associated with higher foveal thickness ($16.33\mu\text{m}$; 95% CI: 5.82 to 26.83 ; $p=0.002$). At the fovea, we found that African-Americans had lower RNFL, GCIPL, INL, and OPL thickness, while male sex was associated with higher foveal GCIPL and ONL thickness. When examining overall macular OCT measures, we found that African-American race was associated with lower INL and OPL thickness, while male sex was associated with higher ONL, IS, OS, and ONLPS thickness. African-American race was also associated with higher pRNFL thickness.

Visual function

AQP4-nonON eyes exhibited worse HCLA (-3.63 letters; 95% CI: -6.13 to -1.13 ; $p=0.004$) and 2.5% LCLA (-6.80 letters; 95% CI: -9.92 to -3.67 ; $p<0.001$) compared to HC (Table 4). 1.25% LCLA was not significantly different between AQP4-nonON eyes and HC (-5.32 letters; 95% CI: -10.75 to 0.11 ; $p=0.055$). Results were similar in sensitivity analyses limited to AQP4-neverON eyes (Suppl. Table 7).

Association of disease duration with OCT measures and VA

In analyses restricted to AQP4-nonON eyes (Suppl. Table 8), disease duration exhibited a negative association with macular RNFL (-0.11 ± 0.05 , $p=0.024$), GCIPL (-0.34 ± 0.08 , $p<0.001$), INL (-0.13 ± 0.04 , $p=0.001$), ONL (-0.29 ± 0.08 , $p<0.001$) and IS (-0.02 ± 0.01 , $p=0.004$), and with pRNFL (-0.45 ± 0.15 , $p=0.003$). At the fovea (Suppl. Table 9), disease duration exhibited a negative association with IS (-0.06 ± 0.02 , $p<0.001$) and a positive association with OPL (0.11 ± 0.05 , $p=0.019$).

Discussion

In the present study, we found quantitative macular retinal layer thickness abnormalities in AQP4-nonON eyes, including both the fovea and surrounding macula, with different patterns of retinal layer involvement by region. More specifically, we found lower ONL, IS, and ONLPS thickness at the fovea and the surrounding macula, and lower macular RNFL and GCIPL thickness. Since chiasmal involvement in AQP4-IgG+ ON is rather common, we performed sensitivity analyses excluding the fellow eyes of patients with unilateral ON

and our results remained largely unaltered.¹ Therefore, our findings are not likely to be due to secondary retrograde changes after symptomatic ON, but may rather relate to subclinical optic neuropathy, a primary retinal process or trans-synaptic degeneration due to subclinical posterior visual pathway involvement. AQP4-nonON eyes also exhibited worse HCLA and LCLA compared to HC, supporting the functional relevance of the observed retinal layer quantitative abnormalities.

The foveal microstructure is of interest in AQP4-IgG+ NMOSD, given the relative abundance of AQP4-expressing Müller glial cells in this region.^{5,7,8} Jeong et al. found lower foveal and inner macular thickness in AQP4-nonON eyes, while Oertel et al. reported lower foveal thickness in AQP4-IgG+ patients with isolated myelitis in the absence of GCIPL or pRNFL thinning.^{9,10} Furthermore, a recent study utilizing foveal morphometry reported wider and flatter fovea in AQP4-IgG+ NMOSD compared to HC.¹¹ In these studies, it was hypothesized that these findings were suggestive of Müller cell dysfunction and a retinal astrocytopathy in NMOSD, and that foveal morphology may serve as a biomarker for early diagnosis.^{10,11} In our study, even though foveal thickness was quantified over the same region, we did not observe the pronounced isolated foveal thinning that was reported in the aforementioned publications; rather, despite our larger sample size, we found similar overall foveal thickness in AQP4-nonON and HC eyes. Nevertheless, our results highlight that the demographic characteristics of the study population are an important consideration when examining foveal OCT measures. This observation is in accordance with prior work, which has shown sex- and race-related differences in the morphology and structure of the fovea, with lower central subfield thickness observed in females and African-Americans, findings that were seen in our study as well.^{18–21} Given the significant sex- and race-related differences in foveal pit morphology (including in HC), these factors should be a consideration in the design of future studies, especially when taking into account the variation in prevalence of NMOSD by race/ethnicity.²²

Our ONL/photoreceptor complex findings are in line with those of You et al., who found lower thickness of the Henle fiber-ONL-IS (HFONL-IS) complex at the fovea in AQP4-IgG+ eyes compared to HC.⁵ The same study reported reduction of the b-wave amplitude in scotopic full-field electroretinography (ERG) that was mainly caused by reduction of the slow PII component, suggestive of Müller cell dysfunction, and the amplitude reduction correlated with the thickness of the HFONL-IS complex. In our study, AQP4-nonON eyes had lower ONL, IS, and ONLPS thickness compared to HC. These findings were not limited to the fovea however, as similar findings were observed in the surrounding macula. Notably, analyses of outer retinal layer thicknesses from macular regions outside of the fovea were not reported by the aforementioned studies evaluating alterations in foveal thicknesses in NMOSD. The ONL finding is in accordance with prior work by our group, in which we found lower ONL thickness in AQP4-ON compared to HC eyes, as well as compared to ON eyes of patients with MS or myelin oligodendrocyte glycoprotein-IgG (MOG-IgG) seropositivity.²³ The pathophysiological mechanisms underlying these findings are not clear, however we speculate that these results are consistent with subclinical primary retinal involvement in AQP4-IgG+ NMOSD via alteration of the dynamics of retinal astrocyte and Müller cell function. In a pathologic study of retinas from patients with AQP4-IgG+ NMOSD, scattered loss of AQP4 immunoreactivity was observed in Müller cells in

the INL, OPL, and ONL.²⁴ Deletion of AQP4 in mice leads to increased susceptibility to light-induced retinal degeneration and osmotic stress, and induces an inflammatory response in the retina.^{25,26} Furthermore, selective ablation of Müller cells in a mouse model led to photoreceptor apoptosis and vasculopathy with breakdown of the blood-retinal barrier.²⁷ Notably, retinal vascular changes have also been reported in fundoscopic and OCT angiography studies in NMOSD, while both active and inactive NMOSD lesions demonstrate prominent vascular fibrosis and hyalinization.^{28–30}

Other studies have also examined if AQP4-IgG+ eyes exhibit evidence of retinal ganglion cell neuronal and axonal loss in the absence of ON, but findings have been conflicting. While some studies reported no differences in GCIPL or pRNFL thickness between AQP4-nonON and HC eyes, Oertel et al. found both lower GCIPL thickness at baseline and accelerated GCIPL atrophy rates in AQP4-nonON eyes compared to HC.^{31–33} Tian et al. found alterations in diffusion tensor imaging measures of the optic radiations (OR) and lower inner retinal thickness in NMOSD (mostly AQP4-IgG+) patients without prior ON compared to HC.³⁴ In the current study, we observed lower RNFL and GCIPL thickness in AQP4-nonON eyes. Importantly, these results remained largely unaltered in analyses limited to patients who had never experienced ON in either eye, suggesting that our findings are not due to carry-over effects from contralateral ON (due to chiasmal or optic tract involvement), but are likely the consequences of processes that are independent of clinically overt ON attacks. Similar to our finding in AQP4-nonON eyes, MS eyes without a history of ON have also been shown to have RNFL and GCIPL atrophy; likely reflecting subclinical inflammation of the visual pathway or retrograde trans-synaptic degeneration.³⁵ However, based on a large recent meta-analysis, the effect size was larger than what was observed in the present study for AQP4-nonON eyes, suggesting that subclinical optic nerve involvement is more extensive or more common in MS.³⁵ Even though subclinical activity is not thought to occur commonly in AQP4-IgG+ disease, gadolinium enhancement of the optic nerve has been reported at the time of attacks involving other anatomical locations, in the absence of visual symptoms.³⁶ Moreover, in the pathologic study of the anterior visual pathway in AQP4-IgG+ NMOSD by Hokari et al, evidence of optic nerve involvement was present in two cases with no history of clinical ON.²⁴

In the current study, we also observed a negative association between disease duration and RNFL, GCIPL, INL, ONL and IS thicknesses, independent of age, with more pronounced retinal layer thinning in patients with longer disease duration. This finding suggests that OCT measures continue to evolve during the disease process. Further longitudinal studies are needed to investigate the evolution of retinal layer thickness over time, both during periods of disease stability and during inflammatory activity.

This study has a number of limitations that warrant discussion. Firstly, brain MRIs were not systematically performed at the time of the OCT scan. Therefore, we cannot exclude the presence of asymptomatic lesions involving the visual pathway, which could be partially responsible for the observed inner retinal layer thinning in AQP4-nonON eyes via retrograde trans-synaptic neurodegeneration. This phenomenon has been previously described in MS, and correlations have been reported between OR lesion volume and RNFL thickness.^{37,38} However, patients with symptomatic cerebral lesions were excluded and OR lesions are

uncommon in NMOSD compared to MS.³⁹ Otherwise, our sample size was relatively low; however, given the rarity of the studied disease, our study is one of the largest to date examining subclinical retinal quantitative abnormalities microstructural changes in AQP4-IgG+ NMOSD eyes without a history of ON. Multi-center studies with larger sample sizes will be important to validate these findings. Furthermore, we were unable to investigate the influence of disease-modifying treatments on OCT measures, given our sample size and the cross-sectional nature of the study. Finally, our study did not incorporate electroretinography, which would be useful to assess for electrophysiological correlates of our structural findings.

In conclusion, the present work suggests that subclinical retinal involvement is present in AQP4-IgG+ eyes without a prior history of ON. We observed findings consistent with retinal ganglion cell neuronal and axonal loss and photoreceptor abnormalities. These findings provide further support to the hypothesis of direct retinal injury outside of events of ON in AQP4-IgG+ NMOSD. Future, larger longitudinal studies will be important to determine the clinical implications of these findings and retinal histopathological studies will be critical to further elucidate the underlying pathoetiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* 2015; 85: 177–189. [PubMed: 26092914]
2. Lennon VA, Kryzer TJ, Pittock SJ, et al. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* 2005; 202: 473–477. [PubMed: 16087714]
3. Ratelade J and Verkman AS. Neuromyelitis optica: Aquaporin-4 based pathogenesis mechanisms and new therapies. *Int J Biochem Cell Biol* 2012; 44: 1519–1530. [PubMed: 22713791]
4. Nagelhus EA, Veruki ML, Torp R, et al. Aquaporin-4 water channel protein in the rat retina and optic nerve: polarized expression in Müller cells and fibrous astrocytes. *J Neurosci* 1998; 18: 2506–2519. [PubMed: 9502811]
5. You Y, Zhu L, Zhang T, et al. Evidence of Müller Glial Dysfunction in Patients with Aquaporin-4 Immunoglobulin G-Positive Neuromyelitis Optica Spectrum Disorder. *Ophthalmology* 2019; 126: 801–810. [PubMed: 30711604]
6. Bringmann A, Pannicke T, Grosche J, et al. Müller cells in the healthy and diseased retina. *Prog Retin Eye Res* 2006; 25: 397–424. [PubMed: 16839797]
7. Kolb H, Nelson RF, Ahnelt PK, et al. The Architecture of the Human Fovea. In: Kolb H, Fernandez E and Nelson R (eds) *Webvision: The Organization of the Retina and Visual System*. Salt Lake City (UT): University of Utah Health Sciences Center, 1995.
8. Distler C and Dreher Z. Glia cells of the monkey retina--II. Müller cells. *Vision Res* 1996; 36: 2381–2394. [PubMed: 8917802]
9. Jeong IH, Kim HJ, Kim N, et al. Subclinical primary retinal pathology in neuromyelitis optica spectrum disorder. *J Neurol* 2016; 263: 1343–1348. [PubMed: 27142716]

10. Oertel FC, Kuchling J, Zimmermann H, et al. Microstructural visual system changes in AQP4-antibody-seropositive NMOSD. *Neurol Neuroimmunol Neuroinflamm* 2017; 4: e334. [PubMed: 28255575]
11. Motamedi S, Oertel FC, Yadav SK, et al. Altered fovea in AQP4-IgG-seropositive neuromyelitis optica spectrum disorders. *Neurol Neuroimmunol Neuroinflamm* 2020; 7.
12. Syc SB, Warner CV, Hiremath GS, et al. Reproducibility of high-resolution optical coherence tomography in multiple sclerosis. *Mult Scler* 2010; 16: 829–839. [PubMed: 20530512]
13. Tewarie P, Balk L, Costello F, et al. The OSCAR-IB consensus criteria for retinal OCT quality assessment. *PLoS ONE* 2012; 7: e34823. [PubMed: 22536333]
14. Schippling S, Balk LJ, Costello F, et al. Quality control for retinal OCT in multiple sclerosis: validation of the OSCAR-IB criteria. *Mult Scler* 2015; 21: 163–170. [PubMed: 24948688]
15. Lang A, Carass A, Hauser M, et al. Retinal layer segmentation of macular OCT images using boundary classification. *Biomedical optics express* 2013; 4: 1133–1152. [PubMed: 23847738]
16. Sotirchos ES, Saidha S, Byraiah G, et al. In vivo identification of morphologic retinal abnormalities in neuromyelitis optica. *Neurology* 2013; 80: 1406–1414. [PubMed: 23516321]
17. Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, et al. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. *Neurology* 2016; 86: 2303–2309. [PubMed: 27225223]
18. Wagner-Schuman M, Dubis AM, Nordgren RN, et al. Race- and sex-related differences in retinal thickness and foveal pit morphology. *Invest Ophthalmol Vis Sci* 2011; 52: 625–634. [PubMed: 20861480]
19. Kelty PJ, Payne JF, Trivedi RH, et al. Macular thickness assessment in healthy eyes based on ethnicity using Stratus OCT optical coherence tomography. *Invest Ophthalmol Vis Sci* 2008; 49: 2668–2672. [PubMed: 18515595]
20. Kimbrough DJ, Sotirchos ES, Wilson JA, et al. Retinal damage and vision loss in African American multiple sclerosis patients. *Ann Neurol* 2015; 77: 228–236. [PubMed: 25382184]
21. Caldito NG, Saidha S, Sotirchos ES, et al. Brain and retinal atrophy in African-Americans versus Caucasian-Americans with multiple sclerosis: a longitudinal study. *Brain* 2018; 141: 3115–3129. [PubMed: 30312381]
22. Kim S, Mealy MA, Levy M, et al. Racial differences in neuromyelitis optica spectrum disorder. *Neurology* 2018; 91: e2089–e2099. [PubMed: 30366977]
23. Sotirchos ES, Filippatou A, Fitzgerald KC, et al. Aquaporin-4 IgG seropositivity is associated with worse visual outcomes after optic neuritis than MOG-IgG seropositivity and multiple sclerosis, independent of macular ganglion cell layer thinning. *Mult Scler* 2019; 1352458519864928.
24. Hokari M, Yokoseki A, Arakawa M, et al. Clinicopathological features in anterior visual pathway in neuromyelitis optica. *Ann Neurol* 2016; 79: 605–624. [PubMed: 26836302]
25. Yuan S, Zhang W, Ding J, et al. Increased sensitivity to retinal light damage in aquaporin-4 knockout mice. *Exp Eye Res* 2009; 89: 119–122. [PubMed: 19450458]
26. Pannicke T, Wurm A, Iandiev I, et al. Deletion of aquaporin-4 renders retinal glial cells more susceptible to osmotic stress. *Journal of Neuroscience Research* 2010; 88: 2877–2888. [PubMed: 20544823]
27. Shen W, Fruttiger M, Zhu L, et al. Conditional Müller cell ablation causes independent neuronal and vascular pathologies in a novel transgenic model. *J Neurosci* 2012; 32: 15715–15727. [PubMed: 23136411]
28. Green AJ and Cree BaC. Distinctive retinal nerve fibre layer and vascular changes in neuromyelitis optica following optic neuritis. *J Neurol Neurosurg Psychiatry* 2009; 80: 1002–1005. [PubMed: 19465415]
29. Huang Y, Zhou L, Zhang Bao J, et al. Peripapillary and parafoveal vascular network assessment by optical coherence tomography angiography in aquaporin-4 antibody-positive neuromyelitis optica spectrum disorders. *Br J Ophthalmol* 2019; 103: 789–796. [PubMed: 30021816]
30. Lucchinetti CF, Mandler RN, McGavern D, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* 2002; 125: 1450–1461. [PubMed: 12076996]

31. Schneider E, Zimmermann H, Oberwahrenbrock T, et al. Optical Coherence Tomography Reveals Distinct Patterns of Retinal Damage in Neuromyelitis Optica and Multiple Sclerosis. *PLoS ONE*2013; 8: e66151. [PubMed: 23805202]
32. Chen Y, Shi C, Zhou L, et al. The Detection of Retina Microvascular Density in Subclinical Aquaporin-4 Antibody Seropositive Neuromyelitis Optica Spectrum Disorders. *Front Neurol*2020; 11: 35. [PubMed: 32117008]
33. Oertel FC, Havla J, Roca-Fernández A, et al. Retinal ganglion cell loss in neuromyelitis optica: a longitudinal study. *J Neurol Neurosurg Psychiatry*2018; 89: 1259–1265. [PubMed: 29921610]
34. Tian D, Su L, Fan M, et al. Bidirectional degeneration in the visual pathway in neuromyelitis optica spectrum disorder (NMOSD). *Mult Scler*2018; 24: 1585–1593. [PubMed: 28823217]
35. Petzold A, Balcer LJ, Calabresi PA, et al. Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol*2017; 16: 797–812. [PubMed: 28920886]
36. Aksoy D, Gokce E, Kurt S, et al. Subclinical Optic Neuritis in Neuromyelitis Optica. *Journal of Neuro-Ophthalmology*2013; 33: 205–207. [PubMed: 23535569]
37. Klistorner A, Graham EC, Yiannikas C, et al. Progression of retinal ganglion cell loss in multiple sclerosis is associated with new lesions in the optic radiations. *Eur J Neurol*2017; 24: 1392–1398. [PubMed: 28799222]
38. Sinnecker T, Oberwahrenbrock T, Metz I, et al. Optic radiation damage in multiple sclerosis is associated with visual dysfunction and retinal thinning--an ultrahigh-field MR pilot study. *Eur Radiol*2015; 25: 122–131. [PubMed: 25129119]
39. Shen T, You Y, Arunachalam S, et al. Differing Structural and Functional Patterns of Optic Nerve Damage in Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorder. *Ophthalmology*2019; 126: 445–453. [PubMed: 30060979]

Figure 1.

Boxplots of macular retinal layer thicknesses (excluding the fovea) in HC and AQP4 non-ON eyes: (a.) TRT (b.) RNFL (c.) GCIPL (d.) ONL (e.) IS (f.) ONLPS

HC: healthy controls; AQP4: aquaporin-4; ON: optic neuritis; TRT: total retinal thickness; RNFL: retinal nerve fiber layer; GCIPL: ganglion cell+inner plexiform layer; ONL: outer nuclear layer; IS: inner photoreceptor segment; ONLPS: outer nuclear layer & photoreceptor segments

Figure 2.

Boxplot of foveal thickness in HC and AQP4 non-ON eyes

HC: healthy controls; AQP4: aquaporin-4; ON: optic neuritis

Figure 3.

Boxplots of foveal retinal layer thicknesses in HC and AQP4 non-ON eyes: (a.) ONL (b.) IS (c.) ONLPS HC: healthy controls; AQP4: aquaporin-4; ON: optic neuritis; ONL: outer nuclear layer; IS: inner photoreceptor segment; ONLPS: outer nuclear layer & photoreceptor segments

Figure 4.

Boxplot of pRNFL in HC and AQP4 non-ON eyes

HC: healthy controls; AQP4: aquaporin-4; ON: optic neuritis; pRNFL: peripapillary retinal nerve fiber layer

Table 1.

Demographic and clinical characteristics

	HC	AQP4-IgG+ NMOSD
Participants, n	77	51
Age in years, mean (SD)	46.4 (1.5)	47.2 (2.1)
Female, n (%)	64 (83%)	45 (88%)
Race, n (%)		
Caucasian-American	39 (51%)	22 (43%)
African-American	33 (43%)	27 (53%)
Asian-American	4 (5%)	2 (4%)
Other	1 (1%)	0 (0%)
Clinical presentation, n (%)		
Isolated TM	-	24 (47%)
Isolated unilateral ON	-	7 (14%)
Isolated brainstem attack	-	2 (4%)
Brainstem attack & TM	-	7 (14%)
Unilateral ON & TM	-	9 (18%)
Unilateral ON & brainstem attack	-	1 (2%)
Unilateral ON & TM & brainstem attack	-	1 (2%)
Disease duration; median (IQR)	-	4 (1–7)
Drug at the time of the scan		
Rituximab	-	34 (67%)
Ecilizumab	-	1 (2%)
Mycophenolate Mofetil	-	4 (8%)
Azathioprine	-	2 (4%)
Methotrexate	-	2 (4%)
None	-	8 (16%)
AQP4-IgG antibody assay		
CBA	-	22 (43%)
ELISA	-	29 (57%)

HC: healthy controls; AQP4-IgG+ NMOSD: aquaporin-4-IgG seropositive Neuromyelitis Optica Spectrum Disorder; SD: standard deviation; TM: transverse myelitis; ON: optic neuritis; IQR: interquartile range; CBA: cell-based assay; ELISA: enzyme-linked immunosorbent assay

Table 2.

Macular retinal layer thicknesses (excluding the fovea), pRNFL thickness, and comparisons between AQP4 non-ON and HC eyes

Layer ^a	HC mean (SD), μm	AQP4 non-ON mean (SD), μm	AQP4 non-ON vs HC	
			Beta (95% CI) ^b	P-value ^b
RNFL ^c	27.1 (3.1)	25.6 (2.6)	-1.34 (-2.35 to -0.33)	0.009
GCIPL ^c	75.6 (4.5)	73.1 (5.9)	-2.44 (-4.26 to -0.62)	0.009
INL ^c	44.3 (2.5)	43.5 (2.7)	-0.64 (-1.55 to 0.27)	0.17
OPL ^c	19.4 (0.5)	19.4 (0.5)	-0.02 (-0.17 to 0.14)	0.83
ONL ^c	67.7 (5.3)	65.6 (5.5)	-1.98 (-3.85 to -0.12)	0.037
IS ^c	19.7 (0.4)	19.5 (0.4)	-0.16 (-0.30 to -0.02)	0.023
OS ^c	24.1 (0.9)	24.1 (1.1)	0.09 (-0.23 to 0.41)	0.58
ONLPS ^c	111.6 (5.9)	109.2 (6.1)	-2.04 (-4.07 to -0.01)	0.048
TRT ^c	310.3 (11.8)	303.6 (14.2)	-6.25 (-10.85 to -1.65)	0.008
pRNFL ^d	93.4 (8.7)	90.6 (9.2)	-2.79 (-5.69 to 0.11)	<i>0.059</i>

^aAll layers were measured at the macula with the exception of the pRNFL

^bDerived from generalized estimating equation models, adjusted for age, sex and race

^cAvailable for 149 HC eyes and 77 AQP4 non-ON eyes

^dAvailable for 151 HC eyes and 83 AQP4 non-ON eyes

HC: healthy controls; AQP4: aquaporin-4; ON: optic neuritis; SD: standard deviation; CI: confidence interval; RNFL: retinal nerve fiber layer; GCIPL: ganglion cell+inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; IS: inner photoreceptor segment; OS: outer photoreceptor segment; ONLPS: outer nuclear layer & photoreceptor segments (ONL + IS + OS); TRT: total retinal thickness at the macula (within an annulus centered at the fovea with a 1 mm internal diameter and a 5 mm external diameter)

Table 3.

Foveal retinal layer thicknesses and comparisons between AQP4 non-ON and HC eyes

Layer	HC mean (SD), μm (n=148 eyes)	AQP4 non-ON mean (SD), μm (n=78 eyes)	AQP4 non-ON vs HC	
			Beta (95% CI) ^a	P-value ^a
RNFL	7.8 (1.1)	7.7 (0.9)	0.01 (−0.29 to 0.31)	0.95
GCIPL	34.0 (11.5)	32.0 (9.6)	−0.69 (−4.16 to 2.77)	0.70
INL	25.8 (6.9)	26.3 (6.9)	1.50 (−0.72 to 3.72)	0.18
OPL	15.9 (3.8)	16.3 (3.8)	0.59 (−0.65 to 1.82)	0.35
ONL	93.0 (11.3)	88.6 (13.6)	−4.01 (−8.00 to −0.02)	0.049
IS	22.9 (0.7)	22.7 (1.0)	−0.32 (−0.60 to −0.03)	0.029
OS	32.2 (1.7)	32.0 (2.2)	−0.23 (−0.91 to 0.44)	0.50
ONLPS	148.1 (11.6)	143.3 (14.0)	−4.57 (−8.65 to −0.49)	0.028
FT	263.7 (22.7)	257.6 (23.7)	−3.55 (−10.97 to 3.87)	0.35

^aDerived from generalized estimating equation models, adjusted for age, sex and race.

HC: healthy controls; AQP4: aquaporin-4; ON: optic neuritis; SD: standard deviation; CI: confidence interval; RNFL: retinal nerve fiber layer; GCIPL: ganglion cell+inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; IS: inner photoreceptor segment; OS: outer photoreceptor segment; ONLPS: outer nuclear layer & photoreceptor segments (ONL + IS + OS); FT: retinal thickness at the fovea (within a circle of a 1 mm diameter centered at the fovea)

Table 4.

Letter acuity scores and comparisons between AQP4 non-ON and HC eyes

Letter acuity score	HC median (IQR) (n=116 eyes)	AQP4 non-ON median (IQR) (n=70 eyes)	AQP4 non-ON vs HC	
			Beta (95% CI) ^a	P-value ^a
100% contrast (HCLA)	59 (55–64)	55 (49–62)	–3.63 (–6.13 to –1.13)	0.004
2.5% contrast (LCLA)	33 (25–37)	24.5 (14–32)	–6.80 (–9.92 to –3.67)	<0.001
1.25% contrast (LCLA)	20 (13.5–27.5)	9 (3–20)	–5.32 (–10.75 to 0.11)	<i>0.055</i>

^aDerived from generalized estimating equation models, adjusted for age, sex and race

HC: healthy controls; AQP4: aquaporin-4; ON: optic neuritis; IQR: interquartile range; CI: confidence interval; HCLA: high-contrast letter acuity; LCLA: low-contrast letter acuity