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*Original Research Paper*

# The loss of macular ganglion cells begins from the early stages of disease and correlates with brain atrophy in multiple sclerosis patients

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## Abstract

**Background:** The importance of neurodegeneration in multiple sclerosis (MS) is increasingly well recognized.

**Objectives:** To evaluate retinal pathology using optical coherence tomography (OCT) and to investigate possible associations between retinal layers' thickness and specific patterns of gray matter volume in patients with a new diagnosis of MS.

**Methods:** A total of 31 patients underwent OCT scans and brain magnetic resonance imaging. In total, 30 controls underwent the same OCT procedure. The association between focal cortical volume and OCT measurements was investigated with voxel-based morphometry (VBM).

**Results:** Compared to controls, patients' macular retinal nerve fiber layer (mRNFL), macular ganglion cell layer (mGCL), macular inner plexiform layer (mIPL), and macular ganglion cell-inner plexiform layer (mGCIPL) thickness were significantly reduced ($p = 0.0009$, $p = 0.0003$, $p = 0.0049$, and $p = 0.0007$, respectively). Peripapillary RNFL (pRNFL) and temporal sector pRNFL (T-pRNFL) did not show any significant changes, although there was a trend toward T-pRNFL thinning ($p = 0.0254$). VBM analysis showed that mGCIPL and pRNFL were significantly correlated with the volume reduction of occipital-parietal cortex ($p < 0.005$).

**Conclusion:** mRNFL, mGCL, and mIPL are significantly reduced in MS patients without concomitant pRNFL thinning. These retinal changes show a significant association with cortical regions that are known to be important for visuospatial performance.

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## Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS), where demyelination, axonal degeneration/loss, and gliosis are the hallmarks of disease.[1]

Although MS is traditionally regarded as a white matter (WM) demyelinating disease, in which axonal loss occurs late during the disease progression, there is evidence indicating that axonal and neuronal loss may be present since early stages of the disease.[1] Moreover, these neurodegenerative aspects play a major role in determining accumulation of permanent physical and cognitive disabilities.[2,3]

Imaging surrogates, such as brain atrophy on magnetic resonance imaging (MRI) and retinal nerve fiber layer (RNFL) thinning on optical coherence tomography (OCT), can be used for prognostic purposes in MS. As mentioned by Galetta et al.,[4] the earliest application of OCT technology in MS was reported by Parisi et al.[5] in 1999. In this study, the RNFL thickness of the eye with a history of optic neuritis (ON) was shown to be reduced compared to the unaffected

eye of the same MS patient and compared to healthy controls' eyes.[5] These data were then confirmed by other works. Particularly, Fisher et al.[6] studied about a hundred patients with a mean disease duration of 8 years, demonstrating not only that the greatest reductions in RNFL thickness was in eyes with a history of acute ON, but also that the unaffected eyes had thinner RNFL compared to controls, suggesting that the occurrence of chronic axonal loss was separate from acute attacks in MS patients. Recently, a multicenter study including more than 800 MS patients and using OCT showed that peripapillary RNFL (pRNFL) thinning, under a specific threshold, is associated with a significantly increased risk of clinical worsening over 5 years.[7] In this study, patients with clinically isolated syndrome (CIS), relapsing-remitting (RR) MS, and progressive MS were included. The patients' mean disease duration was 6.5 years. Besides pRNFL thinning, ganglion cell loss also occurs in MS eyes with and without ON history. The advent of OCT segmentation has enabled estimation of macular ganglion cell layer integrity, especially quantifying the composite thickness of macular ganglion cell-inner plexiform layer (mGCIPL).[8–10]

To date, as summarized by Saidha et al.,[11] OCT research in MS has predominantly focused on characterizing the impact of retrobulbar optic nerve demyelination over proximal axonal and neuronal retinal architecture. The prevailing hypothesis is that optic nerve demyelination results in retrograde axonal degeneration, culminating in ganglion cell death.[12] Until now, little consideration has been given to the possibility that a primary process targeting retinal neurons, independent of optic neuropathy, may also act in MS, in a way that could be analogous to the early damage of the gray matter (GM) compartment in MS.[13,14] A few studies evaluating the ultra-structural changes in the retina have brought further evidence of a possible primary retinal involvement in MS. Indeed, in a post-mortem study conducted in 2010, Green et al.[15] observed retinal atrophy beyond the retinal ganglion cell (RGC) layer in patients affected by MS and hypothesized that outer retina cell loss could be the consequence of a direct immune-mediated process. Moreover, using new spectral-domain OCT (SD-OCT) software algorithms, which allow an in vivo evaluation of individual retinal layers' thickness, further studies have detected outer retinal changes in MS patients.[8,16] Although measurements of intra-retinal layer thickness using OCT have become increasingly prominent in MS research, further studies are needed to better evaluate inner and outer retinal layers' involvement in MS.

Furthermore, OCT measures were shown to have significant correlation with brain parenchymal fraction,[17,18] even though, according to another study, the correlation with GM and WM volume seemed to be inconsistent.[19] In comparison with the RNFL thickness, mGCIPL thickness appears to better correlate with GM volume, which is linked to disability in MS.[17] Recently, two studies have been performed investigating the correlation between OCT findings and cortical atrophy on RR patients. The first group had a mean disease duration of 9 years,[20] while the second group had a mean disease duration of 7 years.[21] These studies respectively found a significant correlation between pRNFL and the visual cortex,[20] and between macular RNFL (mRNFL) and mGCIPL and the insula.[21] Given these interesting results, further studies are needed to better investigate the association between thickness of retinal layers and focal cortical volumes beyond the primary visual system.

Given these premises, aims of this study were (1) to evaluate retinal layers' changes in eyes without history of ON in patients with a new diagnosis of MS in comparison with healthy controls and (2) to investigate possible associations between OCT thickness, in particular pRNFL and mGCIPL, and specific pattern of GM volumes from the very early stages of the disease (mean disease duration ≤ 1 year).

## Materials and methods

### Subjects

A total of 31 patients with a new diagnosis of RR-MS according to the 2010 revised McDonald criteria[22] were consecutively recruited. They underwent clinical assessment, brain MRI, lumbar puncture (LP), ophthalmological assessment, and OCT at baseline. For each recruited patient, the Expanded Disability Status Scale (EDSS) score was assessed at baseline. An age- and sex-matched control group of healthy subjects was also recruited and underwent the same ophthalmological assessment.

On clinical neurological examination, visuospatial abilities were carefully evaluated, with no evidence of impairment requiring further specific investigation. Regarding a potential subclinical ophthalmological involvement, subjects' visual acuity and color vision were evaluated. Eyes with a history of ON were excluded from the OCT studies and individuals with a history of ON in either eye were excluded from the MRI comparison.

All patients with a refractive error greater than 5.0 diopters (D), media opacity, systemic conditions that could affect the visual system, history of ocular

**Table 1.** Principal demographic, laboratory, and clinical characteristics of subjects at baseline.

|  | MS Patients | Controls |
|---|---|---|
| Number of subjects | 31 | 30 |
| Gender (F:M) | 21:10 | 20:10 |
| Age (mean±SD), years | 35.3±11.8 | 34.1±9.6 |
| Disease duration (mean±SD), months | 9±13 | – |
| Intrathecal IgG synthesis, *n* (%) | 22 (71) | – |
| EDSS (median; IQ1–IQ3) score | 2.0; 1.0-3.0 | – |

MS: multiple sclerosis; SD: standard deviation; EDSS: Expanded Disability Status Scale.

trauma, or concomitant ocular diseases (including glaucoma or other known optic neuropathy) were excluded.

The main demographic and clinical characteristics of all recruited patients and controls are summarized in Table 1.

This study was approved by the Institutional Review Board of the Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico (Milan, Italy). All MS patients and control subjects gave their written informed consent for this research before entering the study.

## OCT
An SD-OCT device (Spectralis, Heidelberg Engineering, Germany) was used to evaluate OCT imaging in all patients; both eyes of MS patients and controls. The standard scanning protocol included 61 high-speed B-scans and each scan was approximately 8.5 mm in length and spaced 118 μm apart. All 61 B-scans were acquired in a continuous, automated sequence and covered a $30° × 25°$ area. A minimum of 20 frames were averaged automatically and used to obtain a good quality image. The central fixation target was used to center the raster scan to the fovea. Color-coded retinal thickness maps were generated automatically by the built-in software of the device by applying the Early Treatment Diabetic Retinopathy Study (ETDRS) grid on the fovea and measurements of the retinal thickness were recorded. The ETDRS grid divides the macula into three concentric rings (center, inner, and outer), with the inner ring measuring 1–3 mm and the outer ring measuring 3–6 mm of diameter (referring to a ring with a diameter of 1 mm centered on the fovea). The grid further divides inner

and outer rings into four quadrants (superior, inferior, temporal, and nasal) (Figure 1). All individual retinal layers were measured with the new SD-OCT Automatic Segmentation Explorer mapping software of the device (Heidelberg Engineering, Heidelberg, Germany). Good scan quality and automatic segmentation were assessed prior to the analysis by a trained ophthalmologist, and poor-quality images were rejected. The automated retinal segmentation software was applied to determine thicknesses of the following parameters: macular retinal nerve fiber layer (mRNFL), macular ganglion cell layer (mGCL), macular inner plexiform layer (mIPL), macular inner nuclear layer (mINL), macular outer plexiform layer (mOPL), macular outer nuclear layer (mONL), and photo receptor (PR) thickness, which was defined as a complex extending from the external limiting membrane (ELM) to the retinal pigment epithelial (RPE) band (Figure 1). The ganglion cell layer-inner plexiform layer (mGCIPL) was determined by combining the mGCL and mIPL parameters. For each layer, average thicknesses were calculated within ETDRS grid quadrants and were compared between the two groups. Following the standard protocol, pRNFL thickness was measured with a 12° circular scan around the optic nerve with the activated eye tracker. The global average pRNFL thickness and the temporal sector RNFL thickness (T-pRNFL) were evaluated.

## MRI acquisition
All MS patients underwent a MRI examination on Achieva 3T scanner (Philips, The Netherlands). The acquisition protocol included (1) a three-dimensional (3D) T1-weighted scan (TR, 9.90 ms; TE, 4.61 ms; flip angle, 8°; slices thickness, 1 mm; gap 0); (2) fluid-attenuated inversion recovery (FLAIR) scan (TR, 11,000 ms; TE, 125 ms; flip angle, 90°; slices thickness, 1 mm; gap 0); and (3) a T2-weighted scan (TR, 2492 ms; TE, 78 ms; flip angle, 90°; slices thickness, 4 mm; gap 0). FLAIR-hyperintense lesions were identified by consensus of two independent trained neurologists (M.C. and M.S.). Lesions were outlined using a semi-automated local thresholding contouring software (Jim 7.0, Xinapse System, Leicester, UK, http://www.xinapse.com/) and the lesion load (LL) was estimated.

## Brain volumetrics
All 3D T1-weighted scans were first visually inspected to exclude the presence of macroscopic artifacts. Data were processed using an optimized voxel-based morphometry (VBM) protocol in Statistical Parametric Mapping 8 (SPM8; Wellcome Department of Imaging Neuroscience; www.fil.ion.

**Figure 1.** Spectral-domain optical coherence tomography (SD-OCT) imaging protocol and analysis. First row: after scanning eyes using 61 high-speed B-scans (a), color-coded retinal thickness maps were generated by the built-in software of the device, which then automatically applied the ETDRS grid centered on the fovea (b). The segmentation software of the SD-OCT device automatically detected each retinal layer, including retinal nerve fiber layer, ganglion cell layer (GCL), inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, outer retinal layers, and retinal pigment epithelium (c). Second row: example of GCL thickness analysis. (d) Color-coded GCL thickness map. (e) GCL thickness in each subfield of ETDRS grid. (f) Automatic segmentation of GCL.

ucl.ac.uk/spm/). The scans of patients with history of ON ($n = 4$) were excluded from the VBM analysis in order to minimize the impact of the retrograde degeneration on the cortical atrophy. Segmentation and normalization produced a GM probability map in Montreal Neurological Institute coordinates. To compensate for compression or expansion during warping of images to match the template, GM maps were modulated by multiplying the intensity of each voxel by the local value derived from the deformation field. All data were then smoothed using a 8-mm full width half maximum (FWHM) Gaussian kernel. Then, GM maps were analyzed in SPM8, using a regression model to assess possible associations between patients' regional GM volumes and other variables of interest. We derived for each scan the GM fraction, calculated as the ratio of total GM volume to total intracranial volume (TIV). Age, gender, and LL were always entered as covariates of no interest. For every T-contrast, we applied the family wise error (FWE) correction for multiple comparisons, and we accepted as significant $p$ values $< 0.05$ (corrected at cluster level).

### Statistical analysis

All statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). All comparisons were tested by Student's *t*-tests for paired data (the right eye of each patient was compared with the right eye of an age- and sex-matched control; the left eye of each patient was compared with the left eye of an age- and sex-matched control), with statistical threshold set to $p$ values $< 0.005$ after Bonferroni's correction for multiple comparisons ($\alpha = 0.05/10 = 0.005$).

The normal distribution of data was preliminarily assessed by Shapiro–Wilk test.

### Results

In all, 62 eyes of 31 patients, without impairment of vision, color vision, and visuospatial abilities, were evaluated, together with 60 eyes of 30 controls. Four eyes of four patients were excluded from the analysis due to previous history of ON. The comparison of the mean thickness of each retinal layer and of pRNFL between the two groups is shown in Table 2. Figure 2 describes the difference of the mean pRNFL and mGCIPL thickness in patients compared to controls. Macular RNFL, mGCL, mIPL, and mGCIPL thickness values were significantly reduced in patients compared to controls ($p = 0.0009$, $p = 0.0003$, $p = 0.0049$, $p = 0.0007$), whereas there was no significant difference in mINL, mOPL, and mONL thicknesses ($p > 0.05$). Neither pRNFL nor T-pRNFL was significantly reduced in MS patients, although there was a trend toward T-pRNFL thinning ($p = 0.1367$, $p = 0.0254$, respectively).

**Table 2.** Retinal layers' thickness of both eyes in MS patients and age- and sex-matched controls.

| | pRNFL (µm) | T-pRNFL (µm) | mRNFL (µm) | mGCL (µm) | mIPL (µm) | mINL (µm) | mOPL (µm) | mONL (µm) | PR (µm) | mGCIPL (µm) |
|---|---|---|---|---|---|---|---|---|---|---|
| MS patients (mean±SD) | 93.92±9.65 | 66.00±11.36 | 247±23.56 | 38.19±4.98 | 32.65±3.05 | 34.27±1.78 | 29.83±3.15 | 64.2±7.45 | 66.65±2.43 | 70.89±7.95 |
| Controls (mean±SD) | 97.24±9.72 | 73.02±13.04 | 267±28.90 | 41.03±3.49 | 34.39±2.66 | 34.57±2.11 | 29.71±2.52 | 66.20±5.20 | 65.61±2.12 | 75.46±5.46 |
| *T*-test for paired data | *p*=0.1367 | *p*=0.0254 | *p*=0.0009 | *p*=0.0003 | *p*=0.0049 | *p*=0.5409 | *p*=0.8672 | *p*=0.2992 | *p*=0.9541 | *p*=0.0007 |

MS: multiple sclerosis; SD: standard deviation; pRNFL: peripapillary retinal nerve fiber layer; T-pRNFL: temporal sector pRNFL; mRNFL: macular retinal nerve fiber layer; mGCL: macular ganglion cell layer; mIPL: macular inner plexiform layer; mINL: macular inner nuclear layer; mOPL: macular outer plexiform layer; mONL: macular outer nuclear layer; PR: photo receptors; mGCIPL: macular ganglion cell+inner plexiform layer.

Based on the MRI of the brain and the Talairach atlas, the highest numbers of significant voxels from VBM analyses were located in the parietal-temporal-occipital association area. In particular, mGCIPL showed a significant association ($p_{FWE}$corr at cluster level<0.005) in the peristriate cortex and in the parietal cortex (in particular the angular gyrus) (Figure 3(a)), while pRNFL showed a significant correlation ($p_{FWE}$corr at cluster level<0.005) with the visual association cortex, the peristriate cortex, and the right cerebellar cortex ($p_{FWE}$corr at cluster level<0.05) (Figure 3(b)).

No correlation between OCT measurements and EDSS score at baseline was found. No correlation between OCT measurements and oligoclonal bands (OCB) was found.

## Discussion

In this study, we demonstrated that, at early clinical stages, mRNFL, mGCL, mIPL, and mGCIPL thicknesses were significantly reduced in MS patients compared to controls, whereas there was no difference in pRNFL thickness apart from a trend in T-pRNFL thinning. There are a few possible explanations for this result. First, this finding may be due to a better sensitivity of the ganglion cell bodies (mGCL), dendrites (mIPL/mGCIPL), and axon initial segments (mRNFL) than the peripapillary axons (pRNFL) to identify initial retinal thickness changes in early MS. Indeed, our findings are in line with a recent study conducted in a group of RR-MS patients, which showed that GCIPL measurements had significantly better sensitivity in detecting retinal thinning than temporal pRNFL thickness measured by both Cirrus and Spectralis OCT.[23] Second, ganglion cells' damage could potentially precede pRNFL abnormalities in MS patients. Even though some of the previous studies reported a concomitant thinning of GCIPL and pRNFL in MS, this result was not obtained during the early stages of the disease.[7,17,20,21] Similarly, also in eyes affected by early glaucoma, mGCIPL change has been detected before corresponding pRNFL change in one of the most recent studies.[24] Finally, isolated ganglion cell thinning could reflect a primary process targeting retinal neurons' bodies but not yet affecting the peripapillary axons. In accordance with previous findings,[25] our data indicate that retinal pathology might not only develop as a consequence of inflammatory attacks to the anterior optic pathway, but the retina itself may be a primary target of degenerative processes, possibly in combination with inflammatory mechanisms. Indeed, the retinal damage seems to be more widespread and complex than previously known. Post-mortem studies of MS patients clearly demonstrated localized inflammatory cellular infiltrates surrounding retinal veins in
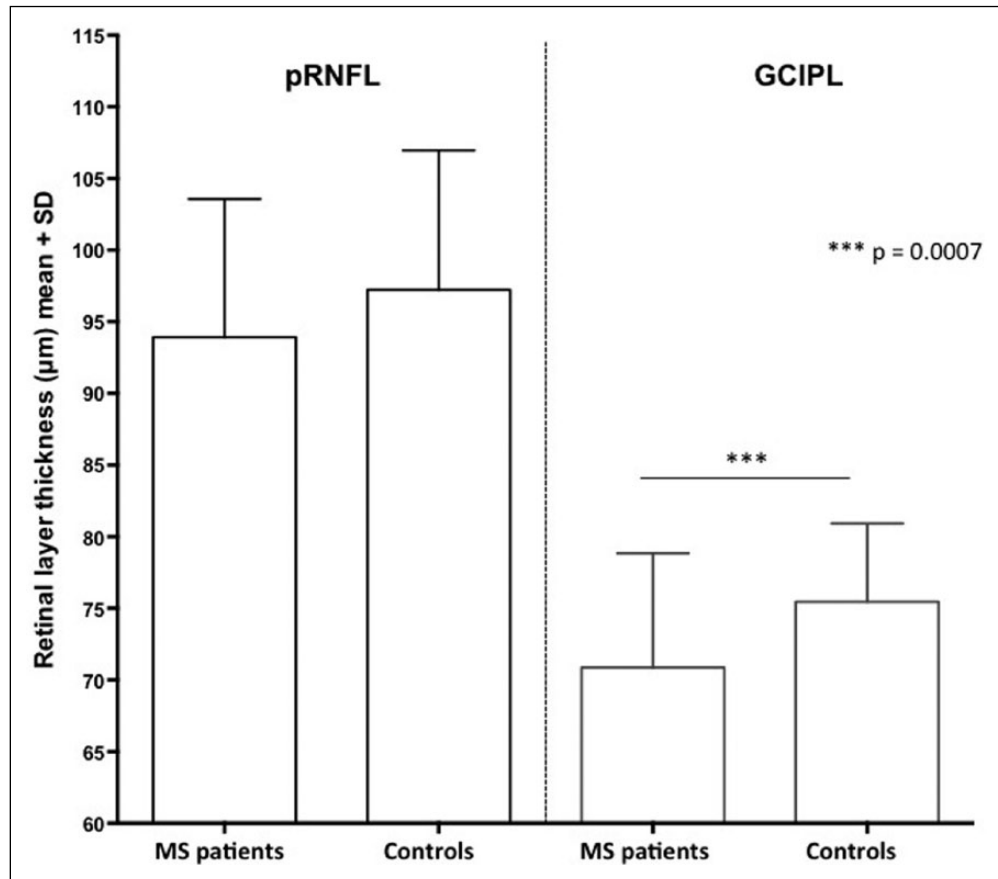
**Figure 2.** Box plots illustrating mean and SD for pRNFL (left) and macular GCIPL (right) thickness in patients compared to controls.

the connective tissue of the retinal nerve fiber layer and RGC layer and atrophy beyond the RNFL in the deeper retinal layers.[15] Nevertheless, in our study, we could not find any retinal changes beyond the inner plexiform layer in MS patients compared to controls.

Regarding the association between OCT thickness and a specific pattern of GM volume, our data suggest that the pattern of cerebral cortical atrophy, particularly in crucial areas for visuospatial performance, is altered in early phases of the disease. In particular, mGCIPL thinning was specifically correlated with posterior visual pathway GM atrophy, defined by a decrease in peristriate cortex, parastriate cortex, and posterior parietal cortex. Surprisingly, pRNFL thickness was not significantly reduced in MS patients compared to controls, but correlated significantly with GM volume in the visual areas. From a pathophysiologic point of view, as speculated by Stellmann et al.,[21] the correlation between OCT outcomes and focal cortical volume might be explained by anterograde and retrograde neurodegeneration within the visual system and its connections to major integrating network hubs.[20] In their work, Stellmann and colleagues cited the so called "second-order effect"

hypothesis by Steenwijk et al.,[26] where focal neurodegeneration leads to increased GM loss in important network hubs along defined anatomical structures. This hypothesis is supported by studies about the hierarchical network architecture in the brain.[27] Within this conceptual framework, it may be assumed that a progressive loss of neurons in cortical hub regions follows neuronal loss in the anterior visual pathway. How far a retrograde loss in the retina might be driven by peripheral or hub atrophy remains speculative.

In our study, VBM analyses detected a closer correlation of ganglion cell layer and inner plexiform layer with cortical volumes than between RNFL and cortical volumes, in line with previous studies that found a better association of mGCIPL with global brain volume measurements.[20]

Certain limitations exist for this study. First, for peripapillary measurements, scans around the optic disk were not acquired using the new spectralis N-site axonal analysis, which provides a correction for foveadisk orientation. However, in all cases, foveal fixation and segmentation were checked to be correct and only
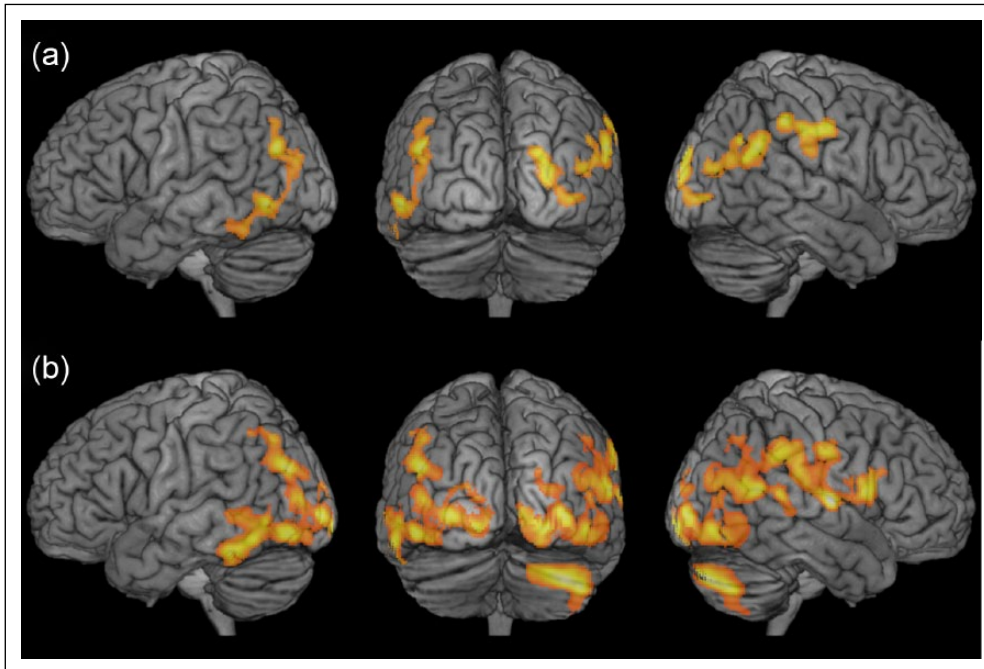
**Figure 3.** (a) VBM analysis showed significant correlation of GM volume with macular GCIPL thickness in the peristriate cortex and in the parietal cortex (in particular, the angular gyrus). $P_{FWE}$corr at cluster level < 0.005 and (b) VBM analysis showed significant correlation of GM volume with pRNFL thickness in the visual association cortex, the peristriate cortex and the right cerebellar cortex. $P_{FWE}$corr at cluster level <0.005.

patients with OCT images without any motion artifact, involuntary saccade, or obvious decentration misalignment were enrolled in the study. Second, although application of retinal segmentation using OCT has become increasingly crucial in MS clinical studies, the approaches used to determine the mean retinal layers' thicknesses vary greatly and insufficient data exist on the reliability of different thickness estimates. A recent study by Oberwahrenbrock et al.[28] proved reliable thickness estimate of the 6-mm-diameter area around the fovea. However, we did not perform a reproducibility study to assess repeatability of the segmentation within the context of our individual study. Third, our current results were obtained from a quite small cohort and may need confirmation in studies on larger patient populations.

In conclusion, in this study, we documented that mRNFL, mGCL, and mIPL are significantly reduced in MS patients already at the time of diagnosis, that is, likely in early, most inflammatory, stages of the disease, without a concomitant pRNFL thinning; this result may suggest that retinal damage could begin from the macular ganglion cells and might not be secondary to the axonal damage, as currently hypothesized.[11] Moreover, OCT outcomes were correlated with patterns of cortical atrophy crucial for visuospatial performance since early stages of the disease.

**References**
1. Frischer JM, Bramow S, Dal-Bianco A, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 2009; 132: 1175–1189.

2. Bjartmar C, Kidd G, Mork S, et al. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. *Ann Neurol* 2000; 48: 893–901.

3. Grzegorski T and Losy J. Cognitive impairment in multiple sclerosis—A review of current knowledge and recent research. *Rev Neurosci*. Epub ahead of print 8 August 2017. DOI: 10.1515/revneuro-2017-0011.

4. Galetta KM, Calabresi PA, Frohman EM, et al. Optical coherence tomography (OCT): Imaging the visual pathway as a model for neurodegeneration. *Neurotherapeutics* 2011; 8(1): 117–132.

5. Parisi V, Manni G, Spadaro M, et al. Correlation between morphological and functional retinal impairment in multiple sclerosis patients. *Invest Ophthalmol Vis Sci* 1999; 40: 2520–2527.

6. Fisher JB, Jacobs DA, Markowitz CE, et al. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. *Ophthalmology* 2006; 113: 324–332.

7. Martinez-Lapiscina EH, Arnow S, Wilson JA, et al. Retinal thickness measured with optical coherence tomography and risk of disability worsening in multiple sclerosis: A cohort study. *Lancet Neurol* 2016; 15(6): 574–584.

8. Behbehani R, Abu Al-Hassan A, Al-Salahat A, et al. Optical coherence tomography segmentation analysis in relapsing remitting versus progressive multiple sclerosis. *PLoS ONE* 2017; 12(2): e0172120.

9. Saidha S, Syc SB, Ibrahim MA, et al. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. *Brain* 2011; 134: 518–533.

10. Saidha S, Syc SB, Durbin MK, et al. Visual dysfunction in multiple sclerosis correlates better with optical coherence tomography derived estimates of macular ganglion cell layer thickness than peripapillary retinal nerve fiber layer thickness. *Mult Scler* 2011; 17: 1449–1463.

11. Saidha S, Sotirchos ES, Oh J, et al. Relationships between retinal axonal and neuronal measures and global central nervous system pathology in multiple sclerosis. *JAMA Neurol* 2013; 70: 34–43.

12. Shindler KS, Ventura E, Dutt M, et al. Inflammatory demyelination induces axonal injury and retinal ganglion cell apoptosis in experimental optic neuritis. *Exp Eye Res* 2008; 87(3): 208–213.

13. Geurts JJ, Bö L and Pouwels PJ. Cortical lesions in multiple sclerosis: Combined postmortem MR imaging and histopathology. *AJNR Am J Neuroradiol* 2005; 26(3): 572–577.

14. Calabrese M, Atzori M, Bernardi V, et al. Cortical atrophy is relevant in multiple sclerosis at clinical onset. *J Neurol* 2007; 254(9): 1212–1220.

15. Green AJ, McQuaid S, Hauser SL, et al. Ocular pathology in multiple sclerosis: Retinal atrophy and inflammation irrespective of disease duration. *Brain* 2010; 133(Pt 6): 1591–1601.

16. Al-Louzi OA, Bhargava P, Newsome SD, et al. Outer retinal changes following acute optic neuritis. *Mult. Scler* 2016; 22(3): 362–372.

17. Balcer LJ, Miller DH, Reingold SC, et al. Vision and vision-related outcome measures in multiple sclerosis. *Brain* 2015; 138(Pt 1): 11–27.

18. Petzold A, De Boer JF, Schippling S, et al. Optical coherence tomography in multiple sclerosis: A systematic review and meta-analysis. *Lancet Neuro* 2010; 9: 921–932.

19. Young KL, Brandt AU, Petzold A, et al. Loss of retinal nerve fibre layer axons indicates white but not grey matter damage in early multiple sclerosis. *Eur J Neurol* 2013; 20: 803–811.

20. Gabilondo I, Martínez-Lapiscina EH, Martínez-Heras E, et al. Trans-synaptic axonal degeneration in the visual pathway in multiple sclerosis. *Ann Neurol* 2014; 75: 98–107.

21. Stellmann JP, Cetin H, Young KL, et al. Pattern of gray matter volumes related to retinal thickness and its association with cognitive function in relapsing-remitting MS. *Brain Behav* 2016; 7(2): e00614.

22. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69(2): 292–302.

23. Gonzales-Lopez JJ, Rebolleda G, Leal M, et al. Comparative diagnostic accuracy of ganglion cell-inner plexiform and retinal nerve fiber layer thickness measures by cirrus and spectralis optical coherence tomography in relapsing-remitting multiple sclerosis. *Biomed Res Int* 2014; 2014: 128517.

24. Kim YK, Ha A, Na KI, et al. Temporal relation between macular ganglion cell inner plexiform layer loss and peripapillary retinal nerve fiber layer loss in glaucoma. *Ophthalmology* 2017; 124: 1056–1064.

25. Brandt AU, Oberwahrenbrock T, Ringelstein M, et al. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. *Brain* 2011; 134(Pt 11): e193.

26. Steenwijk MD, Geurts JJ, Daams M, et al. Cortical atrophy patterns in multiple sclerosis are non-random and clinically relevant. *Brain* 2016; 139(Pt 1): 115–126.

27. Filippi M, van den Heuvel MP, Fornito A, et al. Assessment of system dysfunction in the brain through MRI-based connectomics. *Lancet Neurol* 2013; 12: 1189–1199.

28. Oberwahrenbrock T, Weinhold M, Mikolajjczak J, et al. Reliability of intra-retinal layer thickness estimates. *PLoS ONE* 2015; 10(9): e0137316.