Astrocytic outer retinal layer thinning is not a feature in AQP4-IgG seropositive neuromyelitis optica spectrum disorders

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ABSTRACT

Background Patients with anti-aquaporin-4 antibody seropositive (AQP4-IgG+) neuromyelitis optica spectrum disorders (NMOSDs) frequently suffer from optic neuritis (ON) leading to severe retinal neuroaxonal damage. Further, the relationship of this retinal damage to a primary astrocytopathy in NMOSD is uncertain. Primary astrocytopathy has been suggested to cause ON-independent retinal damage and contribute to changes particularly in the outer plexiform layer (OPL) and outer nuclear layer (ONL), as reported in some earlier studies. However, these were limited in their sample size and contradictory as to the localisation. This study assesses outer retinal layer changes using optical coherence tomography (OCT) in a multicentre cross-sectional cohort.

Method 197 patients who were AQP4-IgG+ and 32 myelin-oligodendrocyte-glycoprotein antibody seropositive (MOG-IgG+) patients were enrolled in this study along with 75 healthy controls. Participants underwent neurological examination and OCT with central postprocessing conducted at a single site. No significant thinning of OPL (25.02±2.03 µm) or ONL (63.59±5.78 µm). Longitudinal studies are necessary to determine if OPL and ONL are damaged in late disease due to retrograde trans-synaptic degeneration and whether outer retinal dysfunction occurs despite any measurable structural correlates.

INTRODUCTION

Neuromyelitis optica spectrum disorders (NMOSDs) are relapsing autoimmune disorders affecting the central nervous system (CNS).1 Common clinical attacks in NMOSD include optic neuritis (ON), acute myelitis and area postrema syndrome.2 Serum autoantibodies to aquaporin-4 (AQP4-IgG) are detectable in 60%–80% of patients with NMOSD.3,4 AQP4 is an astrocytic water channel in the CNS.5 In the retina, astrocytes are mainly located in the inner neuroaxonal layers of the retina, but AQP4 damage is additionally highly expressed in retinal Müller cells.6 These glial cells have diverse functions, such as regulation of water homeostasis and neurotransmitter recycling, and are located around the fovea.
on the exact layers in which these changes occur. It thereby remains unclear if ORLs, especially the ONL, are also potentially affected by primary retinal astrocytopathy in AQP4-IgG seropositive NMOSD.

Representing the largest international NMOSD dataset collected so far, the CROCTINO study (Collaborative Retrospective Study on retinal optical coherence tomography (OCT) in Neuromyelitis Optica) overcomes one of the common weaknesses of NMOSD studies—being limited to small and homogeneous sample populations. Using OCT data from over 20 centres worldwide, reliable quantitative and qualitative retinal assessment becomes possible, and controversial questions such as ORL changes in AQP4-IgG seropositive NMOSD can be clarified. Apart from patients who were AQP4-IgG seropositive, the CROCTINO cohort also includes patients with antibodies to myelin-oligodendrocyte-glycoprotein (MOG-IgG), a group that is now believed to be a distinct disease entity. While clinically similar and undergoing comparable retinal neurodegeneration after ON, MOG-IgG-associated disease (MOGAD) lacks an identifiable astrocytopathy component and is thereby an appropriate diseased control group for patients who were AQP4-IgG seropositive when investigating astrocytic changes.

In this study, we investigated if ORL thinning, specifically in the foveal and macular ONL, occurs in patients who were AQP4-IgG seropositive compared with healthy controls (HCs) and with patients with MOGAD as a diseased control group.

**METHODS**

**Cohort design**

A total of 539 patients with NMOSD were recruited between 2000 and 2018 as part of CROCTINO (stratified data of centres by device type and number of patients are summarised in the online supplemental file). Patients with (1) diseases potentially confounding OCT analyses (including glaucoma, diabetic retinopathy, retinal surgery and ametropia greater than ±6 diopters), (2) a history of ON within the last 6 months before baseline, (3) no evidence of seropositivity for AQP4-IgG or MOG-IgG and (4) no macular OCT data were excluded. Cell-based assays were used for the detection of AQP4-IgG and MOG-IgG antibodies in serum samples from all patients. Clinical data (antibody serology, disease duration, frequency of ON, location of ON, date of ON, Expanded Disability Standard Scale and treatment received) were collected from all patients. We also included 75 HCs (recruited from Barcelona, Isfahan, Mangalore and Berlin), who were neither age nor sex matched to either cohort.

**Optical coherence Tomography**

Retinal examinations were conducted at each centre using the following OCT devices: Spectralis SD-OCT, Heidelberg Engineering, Heidelberg, Germany (Spectralis), Cirrus HD-OCT, Carl Zeiss Meditec Inc, Dublin, California, USA (Cirrus) and Topcon 3D-OCT, Topcon Corp, Tokyo, Japan (Topcon). With respect to each device and each centre, two scans were collected: (1) a 3.4 mm diameter peripapillary ring scan around the optic nerve head for Spectralis SD-OCT (for Cirrus and Topcon devices: extracted from optic disc volume scans), and (2) a macular volume scan, centred on the fovea. Scans were categorised and uploaded onto a central server to be accessed for further processing.

All OCT images fulfilled the OSCAR-IB criteria (see figure 1—images from 29 patients not fulfilling these criteria were excluded) and results were presented in line with the

**Figure 1** Cohort design and exclusion criteria: from the original 539 patients recruited in the CROCTINO cohort, 108 patients were excluded due to missing macular data. Of the remaining 431 patients in the segmentation cohort, a further 40 patients were excluded due to anomalies in their OCT scans (OSCAR-IB criteria; primarily due to low image quality (26 patients) or the presence of microcysts (3 patients) or other pathologies) or due to data corruption (11 patients). We also excluded patients with unknown antibody status (90 patients). Of the remaining 301 patients, the cohort was split based on AQP4-IgG or MOG-IgG seropositivity and a further set of exclusion criteria were applied based upon age (being ≥65 years), ophthalmological comorbidities (eg, glaucoma) and in instances where follow-ups occurred within 6 months of an ON attack. AQP4-IgG, anti-aquaporin-4 antibody; HC, healthy control; MOG-IgG, anti-myelin-oligodendrocyte-glycoprotein antibody; OCT, optical coherence tomography; ON, optic neuritis.
Table 1 Demographic overview

<table>
<thead>
<tr>
<th>Subjects (N)</th>
<th>HC</th>
<th>AQP4-IgG</th>
<th>MOG-IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of eyes (N)</td>
<td>148</td>
<td>317</td>
<td>55</td>
</tr>
<tr>
<td>Age, years, mean±SD</td>
<td>32.3±9.6</td>
<td>41.8±12.1</td>
<td>36.5±13.7</td>
</tr>
<tr>
<td>Sex, male, N (%)</td>
<td>25 (33.8)</td>
<td>24 (12.2)</td>
<td>10 (31.2)</td>
</tr>
<tr>
<td>EDSS (median, IQR)</td>
<td>–</td>
<td>3.5 (2.0–5.0)</td>
<td>2.0 (1.5–2.5)</td>
</tr>
<tr>
<td>Average age at onset (years, median IQR)</td>
<td>–</td>
<td>32.9 (24.9–42.4)</td>
<td>30.0 (17.6–42.5)</td>
</tr>
<tr>
<td>Patients with a history of ON (N %)</td>
<td>–</td>
<td>142 (72.1)</td>
<td>24 (75.0)</td>
</tr>
<tr>
<td>Median number of ON episodes (median, IQR)</td>
<td>–</td>
<td>1.00 (0.00–3.00)</td>
<td>2.00 (1.00–4.00)</td>
</tr>
</tbody>
</table>

Table 2 Group comparison between HC and patients who were AQP4-IgG and MOG-IgG seropositive at baseline (Spectralis devices only)

<table>
<thead>
<tr>
<th>Number of eyes</th>
<th>HC</th>
<th>AQP4-IgG</th>
<th>MOG-IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRNFL in µm (mean±SD)</td>
<td>99.17±9.76</td>
<td>78.48±6.84</td>
<td>74.33±23.44</td>
</tr>
<tr>
<td>mRNFL in µm (mean±SD)</td>
<td>35.25±3.13</td>
<td>28.09±6.50</td>
<td>27.62±5.43</td>
</tr>
<tr>
<td>GCP in µm (mean±SD)</td>
<td>80.62±164</td>
<td>65.81±13.03</td>
<td>66.16±11.85</td>
</tr>
<tr>
<td>INL in µm (mean±SD)</td>
<td>39.64±2.51</td>
<td>39.85±3.57</td>
<td>41.55±4.14</td>
</tr>
<tr>
<td>OPL in µm (mean±SD)</td>
<td>24.52±1.64</td>
<td>25.02±2.03</td>
<td>25.10±2.00</td>
</tr>
<tr>
<td>OLN in µm (mean±SD)</td>
<td>63.59±5.78</td>
<td>61.63±7.04</td>
<td>64.71±7.87</td>
</tr>
<tr>
<td>OPNL in µm (mean±SD)</td>
<td>89.23±6.95</td>
<td>86.65±7.21</td>
<td>89.81±8.61</td>
</tr>
<tr>
<td>PR in µm (mean±SD)</td>
<td>80.80±3.28</td>
<td>80.35±2.94</td>
<td>81.49±3.59</td>
</tr>
<tr>
<td>RT in µm (mean±SD)</td>
<td>324.57±13.24</td>
<td>300.76±20.11</td>
<td>306.6±17.99</td>
</tr>
</tbody>
</table>

AQP4-IgG, anti-aquaporin-4 antibody; B, estimate; GCP, ganglion cell and inner plexiform layer; HC, healthy control; INL, inner nuclear layer; MOG-IgG, anti-myelin-oligodendrocyte-glycoprotein antibody; mRNFL, macular retinal nerve fibre layer; OPL, outer plexiform layer; OLN, outer nuclear layer; OPL, outer plexiform layer; OPNL, outer plexiform and nuclear layer; PR, photoreceptor layer; pRNFL, peripapillary retinal nerve fibre layer; RT, total retinal thickness.

Statistical methods

Data were stratified in cohorts by antibody status and ON condition status (contralateral eyes of patients with a history of unilateral ON are classified not fulfilling the ON history criteria). The data were further bifurcated by OCT device (Spectralis, Cirrus or Topcon) to mitigate any device-specific aberrations. For continuous cohort data (age, average age at onset and disease duration) on each of the AQP4-IgG, MOG-IgG and HC cohorts, the Student’s t-test was employed. Cross-sectional group comparisons of the OCT values were conducted using linear mixed-effect models with age and sex as fixed and centre and patient-ID as random effects; where necessary, models were corrected for age and sex. Marginal and conditional coefficients of determination for the models were estimated by pseudo-$R^2$ for mixed-effect models. Significance was established at $p<0.05$. Statistical
analyses were conducted using R (V.4.0.0) (RStudio Inc, Boston, Massachusetts, USA).27

RESULTS

Cohort description

In total, 197 patients who were AQP4-IgG seropositive fulfilled the inclusion criteria (figure 1, table 1). We also included 75 unmatched HCs and 32 patients who were MOG-IgG seropositive control groups.

Neuroaxonal damage measured by pRNFL, mRFNL and GCIP was comparable in patients who were AQP4-IgG seropositive (pRNFL: 78.46±24.13 µm, mRNFL: 28.09±6.60 µm, GCIP: 65.81±13.03 µm) and MOG-IgG seropositive (pRNFL: 74.33±23.44 µm, mRNFL: 27.62±5.43 µm, GCIP: 66.16±11.85 µm) making MOGAD a highly relevant comparator group for our investigation of ORLs (table 2).

Limited outer retinal changes in AQP4-IgG seropositive NMOSD

No significant thinning of macular OPL and ONL in patients who were AQP4-IgG seropositive (irrespective of ON status) were observed compared with HC or patients who were MOG-IgG seropositive using the 5 mm diameter macular data (table 2, figure 2). No significant changes were observed when the OPL and ONL values were analysed as the combined OPNL. Previous studies described ORL thinning only in the foveal and parafoveal area as a sign of AQP4-IgG-induced Müller cell damage.8 11 We therefore repeated our analyses in both 3 mm and the 1 mm diameter volumes around the fovea, but these narrower volumes showed again no relevant OPL or ONL thinning in patients who were AQP4-IgG seropositive compared with HC or patients who were MOG-IgG seropositive (see online supplemental data). Additionally, while these previous studies reported changes in the inner segment layer of the photoreceptors, this was not seen in our study.8 11

After a previous description11 of ORL changes in patients who were AQP4-IgG seropositive with a history of ON, we also examined ORL differences separately in eyes with a history of ON. AQP4-IgG seropositive eyes with a history of ON (AQP4-ON) did not display any thinning of ONL and OPL compared with patients without a history of ON (AQP4-NON) or HC, despite severe neuroaxonal loss measured by pRNFL and GCIP layer (table 3, figure 3). Comparing patients who were AQP4-IgG and MOG-IgG seropositive, both groups had a comparable neuroaxonal loss (pRNFL, GCIP)—in the whole group as well as in respect of ON and non-ON eyes (table 2, figure 2). AQP4-ON (B=−1.54, SE=0.69 µm, p=0.027) as well as MOG-ON (B=−2.51, SE=0.87 µm, p=0.004) showed an OPL thinning in the fovea (1 mm diameter) compared with HC, but no difference was observed between AQP4-ON and MOG-ON (p=0.100). Also, no significant correlation between ethnicity and current therapies on outer retinal thickness was found (data not shown).

DISCUSSION

Our study suggests that neither macular OPL nor ONL loss occurs in AQP4-IgG seropositive NMOSD, regardless of ON phenotype, as compared with HC and patients who were MOG-IgG seropositive. The MOG-IgG cohort presented a unique opportunity to contrast our AQP4-IgG seropositive cohort with a highly relevant comparator group, which most likely has no astrocytopathy-component.28

Our results differ from those published by You et al in 20198 and Filippatou et al in 2020.11 In both studies, thinning was observed in the ONL and the inner segment of the photoreceptor layers. In the case of You et al, who utilised Spectralis SD-OCT devices for the image acquisition, foveal thinning was observed along with a reduction in b-wave amplitudes in full-field electroretinography (ERG) suggestive of Müller cell dysfunction.8 Filippatou et al, who employed Cirrus-SD-OCT for the image acquisition, also described thinning of the fovea in the 5 mm diameter macular area around the fovea.11 Both studies suggested the ORL changes to be caused by a primary retinal astrocytopathy with AQP4-IgG associated glial dysfunction in Müller cells.29 These pathological responses could account for the associated thinning observed in the ONL in these studies. However, other exogenous factors...
Table 3 OCT results in patients who were AQP4-IgG seropositive stratified by history of ON (Spectralis devices only)

<table>
<thead>
<tr>
<th>ON vs AQP4-NON</th>
<th>ON vs HC</th>
<th>ON vs MOG-ON</th>
<th>AQP4-NON vs MOG-ON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of eyes</td>
<td>232</td>
<td>85</td>
<td>43</td>
</tr>
<tr>
<td>pRNFL in µm (mean±SD)</td>
<td>72.84±24.47</td>
<td>96.09±12.99</td>
<td>68.03±22.95</td>
</tr>
<tr>
<td>85</td>
<td>96.93±17.99</td>
<td>68.15±22.95</td>
<td>61.45±11.96</td>
</tr>
<tr>
<td>43</td>
<td>95.33±7.32</td>
<td>75.06±11.78</td>
<td>70.43±11.78</td>
</tr>
<tr>
<td>12</td>
<td>55.18±5.37</td>
<td>54.18±5.37</td>
<td>54.18±5.37</td>
</tr>
<tr>
<td>62</td>
<td>3.37 &lt;0.001 3.37 &lt;0.001 3.37 &lt;0.001</td>
<td>0.00 0.00 0.00</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>0.75 0.25 0.25</td>
<td>0.25 0.25 0.25</td>
<td>0.25 0.25 0.25</td>
</tr>
<tr>
<td>P</td>
<td>0.275 0.425 0.425</td>
<td>0.425 0.425 0.425</td>
<td>0.425 0.425 0.425</td>
</tr>
<tr>
<td>OPL in µm (mean±SD)</td>
<td>62.94±12.73</td>
<td>77.11±7.56</td>
<td>63.45±11.96</td>
</tr>
<tr>
<td>ONL in µm (mean±SD)</td>
<td>87.58±7.67</td>
<td>87.85±6.78</td>
<td>91.78±6.69</td>
</tr>
<tr>
<td>OPNL in µm (mean±SD)</td>
<td>80.89±2.93</td>
<td>79.80±2.94</td>
<td>79.80±2.94</td>
</tr>
</tbody>
</table>

and OPL. Should the blood–retina barrier be compromised deep vascular plexus intercepts the boundary between the INL and OPL, thereby mediating glial dysfunction in the Müller cells. This may have been what was observed in the OPL from the 1 mm AQP4-ON and MOG-ON cohort given the relative location of the OPL to the INL. To that end, while disease duration did not reveal to any correlates with OPL (p=0.805) or ONL (p=0.833) values, we cannot exclude time-dependent effects in a cross-sectional analysis. We believe that this area warrants more research to quantify if (1) age is a factor, (2) ON damages the barrier function and (3) the INL does indeed play a role as a dam to retrograde axonal degeneration in NMOSD.

A strength of our study rests on its cohort size and composition, which mirrors that of a global population. This result derives from a consortium of expert NMOSD researchers enabling the enrolment of participants through a multicentre strategy. This approach was designed to overcome many of the earlier NMOSD study limitations, for example small and homogeneous sample populations. Additionally, the use of differing OCT devices compounds complexities in OCT comparisons and a high degree of caution is needed in order to rely on differing platforms interchangeably. Thus, our study focuses on use of
The need to focus primarily on the inner layers, particularly the ORLs when tracking disease progression and reinforces in NMOSD, it potentially alleviates the burden of monitoring changes can be observed on account of a primary astrocytopathy damage that have not previously been recognised; as no ORL into relationships between retinal layer changes and axonal

6 Lu A

CONCLUSION
Our results show no evidence of macular ORL changes as a major component of retinal damage in patients who were seropositive AQP4-IgG NMOSD and patients with MOGAD. Further studies will be necessary to clarify (1) if OPL and ONL are damaged in late disease stages due to retrograde trans-synaptic axonal degeneration across the damaged INL barrier and (2) if outer retinal dysfunction without a measurable structural correlate occurs. Longitudinal studies could help quantify changes in the ORLs alongside disease progression.

three widely available OCT devices, and obtained confirmatory results with each of them; of these, two were also employed respectively in the studies by You et al and Filippatou et al.

Limitations of the current study should also be considered. First, the HCs and patients with MOGAD were not matched, which makes it difficult to rule out age-related and gender-related affects. Notably, retinal thickness decreases with age and males generally exhibit higher GCIP and RT. Also, no ERG or functional visual pathway assessments were conducted, which could have potentially shown more subtle functional impairment of ORLs without associated tissue loss. Outer retinal studies are additionally complicated by Henle Fibre morphologies as OCT beam placement plays a major role in how this layer is depicted; the high level of irregularity and variability in these morphologies add a level of subjectiveness in the quantification and correction of outer layer segmentation and analyses. Finally, Cirrus and Topcon measurements could not be utilised as confirmatory cohorts as there lacked sufficient HCs examined with these devices. Nonetheless, the current findings provide insights into relationships between retinal layer changes and axonal damage that have not previously been recognised; as no ORL changes can be observed on account of a primary astrocytopathy in NMOSD, it potentially alleviates the burden of monitoring the ORLs when tracking disease progression and reinforces the need to focus primarily on the inner layers, particularly the RNFL and the GCIP layer.

Figure 3 OCT results stratified by ON status (tested with Spectralis devices): boxplots of mean OCT values with individual eyes (jitter) in HC (left, green), AQP4-IgG cohort (middle) and MOG-IgG cohort (right). Seropositive patients with a history of ON are highlighted with light yellow and seropositive patients without a history of ON are highlighted in orange. (A) pRNFL; (B) GCIP; (C) INL; (D) OPL; (E) ONL; and (F) PR. AQP4, aquaporin-4; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; MOG, myelin-oligodendrocyte-glycoprotein; OCT, optical coherence tomography; ONL, outer nuclear layer; OPL, outer plexiform layer; PR, photoreceptor layer; pRNFL, peripapillary retinal nerve fibre layer.

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Figure 3 OCT results stratified by ON status (tested with Spectralis devices): boxplots of mean OCT values with individual eyes (jitter) in HC (left, green), AQP4-IgG cohort (middle) and MOG-IgG cohort (right). Seropositive patients with a history of ON are highlighted with light yellow and seropositive patients without a history of ON are highlighted in orange. (A) pRNFL; (B) GCIP; (C) INL; (D) OPL; (E) ONL; and (F) PR. AQP4, aquaporin-4; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; MOG, myelin-oligodendrocyte-glycoprotein; OCT, optical coherence tomography; ONL, outer nuclear layer; OPL, outer plexiform layer; PR, photoreceptor layer; pRNFL, peripapillary retinal nerve fibre layer.
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Contributors The study was conceived and designed by AL, HZ, FCO, AB and FP. AL, HZ, FCO, CB, SM and SVs collected and analysed data for the study. HZ, FCO, AB and FP contributed to the scientific design and conduct of the study. AL and FCO performed biostatistical analyses to the study, were responsible for literature research and wrote the manuscript. AP, AIG, MRV, LC and TJS contributed to the conception and design of the study. All other authors contributed to the acquisition and analysis of data. All authors approved the final version of the manuscript.

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Competing interests HZ reports grants from Novartis and speaking honoraria from Bayer Healthcare, unrelated to this study. EHM-L received funding from the Instituto de Salud Carlos III (Spain) and Fondo Europeo de Desarrollo Regional (FEDER-JR16/00006), Grant for MS Innovation, Fundación Privada Cellex and Marató TV3 Charitable Foundation and is a researcher in the OCTIMS study, an observational study (that involves no specific drugs) to validate SD-OCT as a biomarker for MS, sponsored by Novartis, and has received honoraria and travel support for international and national meetings over the last 3 years from Biogen, Novartis, Roche, Genentech. He is a member of the working committee of International Multiple Sclerosis Visual System (IMSVISUAL) Consortium. MAL-P has received funding for travel and speaker honoraria from Novartis, Sanofi-Genezyme and Roche. MAF has nothing to disclose. Jacqueline Palace has received support for scientific meetings and honorariums for advisory work From Merck Serono, Novartis, Chugai, Alexion, Roche, Medimmune, Genentech, UCB, Mitsubishi, Ample, Janssen. Grants from Alexion, Amplbiotechnology. Shares in AstraZeneca. Acknowledges Partial funding by HBO 2015 approved by the UK Department of Health and Social Care. Malva has received compensation for advisory board, consulting, meeting attendance and speaking from Biogen, Teva, and Roche and has travel expenses to scientific meetings sponsored by Bayer HealthCare, Merck Serono, TEVA and Roche. JH has received grants for OCT research from the Förderverband Stiftung and Merck, personal fees and non-financial support from Celgene, Merck, Alexion, Novartis, Roche, Santhera, Biogen, Heidelberg, Engineering, Sanofi Genezyme and non-financial support of the Guthy-Jackson Charitable Foundation, all outside the submitted work. MZ has received research support from the German Federal Ministry of Education and Research (DIFUTURE), Grant Numbers 01ZV1312 and 01ZV1611. He received honoraria for consultancy and travel grants from Merck, Roche, Biogen and for speaking activities from Teva; research support from Merck, Novartis; travel support from Merck, Roche, Biogen, Almirall. MP has nothing to disclose. OA has received honoraria for speaking/consultation and travel grants from Bayer Healthcare, Biogen Idec, Chugai, Novartis, Medimmune, Merck Serono, and Teva and research grants from Bayer Healthcare, Biogen Idec, Novartis, and Teva. MR has received speaker honoraria from Novartis, Bayer, Roche, Alexion and Ipsen and travel reimbursement from Bayer, Merck, Genzyme, Teva, Roche and Merck, none related to this study. PA reports grants, personal fees and non-financial support from Allergan, Biogen, Ipsen, Merz Pharmaceuticals, Novartis, and Roche, personal fees and non-financial support from Bayer Healthcare, and Merck, and non-financial support from Sanofi-Aventis/Genezyme. HJK reports speaking and/or consulting; Bayer Schering Pharma, Biogen, Celltrion, Esaïe, HanAll Biopharma, Meira, Novartis, Sanofi, Serono, and UCB; research support: Ministry of Science & ICT, Sanofi Genzyme, Teva-Handok, and UCB; steering committee member: Medimmune; co-editor/associated editor: MS Journal-Experimental, Translational and Clinical; and Journal of Clinical Neurology. J-WH has received a grant from the National Research Foundation of Korea. YMD has served as a consultant and/or received grant support from: Accorda, Bayer, Pharmaceutical, Biogen Idec, Celgene, EMD Serono, Genzyme, Novartis, Questor, Chugai, and Teva Neuroscience and is currently supported by grants from NIH NIAID Autoimmune Center of Excellence: UM1-A110557; NIH NINDS R01-N5080281. HSK has nothing to disclose. IK served on scientific advisory board for Biogen Idec and Genentech and received research support from Guthy-Jackson Charitable Foundation, National Multiple Sclerosis Society, Biogen Idec-Serono, Genzyme and Novartis. ZR has nothing to disclose. He has received research support from the German Association of Neurology (Deutsche Gesellschaft für Neurologie) in context of project.CC has received a speaking honorarium from Bayer and guest research funding from Novartis unrelated to this publication. All other authors have nothing to disclose.

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the Declaration of Helsinki (1964) in its currently applicable version at their own discretion and in accordance with relevant local laws. The study also conformed to all relevant best practice guidelines and ethical standards of each centre.

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