SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

Optical coherence tomography (OCT) eligibility criteria

The following criteria were used to determine which participants to include in the OCT analyses. Participants with a history of diabetes mellitus, uncontrolled hypertension, glaucoma, prior ocular surgery or trauma, refractive errors exceeding ±6 diopters, or other significant neurological or ophthalmological conditions were excluded. Data from eyes that developed optic neuritis (ON) during the course of the study were censored at the time of the last available OCT prior to the onset of ON, and data from eyes with ON within six months of baseline OCT were excluded, in order to mitigate the potential effects of acute ON on rates of retinal layer atrophy.

OCT scanning and processing

All scans were reviewed by experienced technicians and any scans with signal strength below 7/10, or with artifact, were excluded, in accordance with the OSCAR-IB criteria.[1,2] Peri-papillary retinal nerve fiber layer (pRNFL) thickness values were generated by conventional Cirrus HD-OCT software. Segmentation of the ganglion cell + inner plexiform layers (GCIPL) was performed utilizing an automated segmentation algorithm which calculates the average GCIPL thickness within an annulus, centered at the fovea, with an internal diameter of 1mm and an external diameter of 5mm.[3] All macular cube scans were reviewed to confirm the accuracy of the segmentation.

Blood collection, lipid extraction and liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) analysis

The study participants underwent phlebotomy and the blood was processed using our standard protocol, with serum aliquots stored at -80°C until the time of lipidomics analyses.[4] Targeted lipidomics analyses were performed, which involved the quantification of several circulating sphingolipids. These included ceramides, dihydroceramides, hexosyl-ceramides and lactosyl-ceramides. Lipids were extracted from serum samples using a modified Bligh&Dyer method.[5] Briefly, internal standards ceramide (d18:1/12:0) (Avanti Polar Lipids, Alabaster, AL, USA) were added to the extraction solvent at a concentration of 1.3 µg/mL.[6] After the clear phase separation, organic layers containing crude lipid extracts were collected and dried in a nitrogen evaporator (Organomation, Berlin, MA, USA) and stored at -80°C. Dried extracts were resuspended in pure methanol prior to analysis.

Ceramide analyses

Chromatographic separations of ceramide were performed on a C18 reverse-phase column (2.6 µm, 50x2.1 mm) with an ULTRA HPLC In-Line Filter (0.5 µm Depth Filter x 0.004 in ID) (Phenomenex, Torrance, CA, USA) using a Shimadzu ultra-fast liquid chromatography (UFLC) system (Shimadzu, Nakagyo-ku, Kyoto, Japan) coupled to a triple quadrupole mass spectrometer (API3000, SCIEX, Thornhill, ON, Canada). Electrospray Ionization (ESI, +ve) was used to ionize these lipid species and individual ceramide species were detected by multiple reaction monitoring (MRM). Mass spectrometry conditions and HPLC parameters were similar to those described in previous study.[7] In order to monitor the instrument condition over the run of samples, quality control (QC) samples were injected in every 10 injections. Eight-point calibration curves (0.1–1000 ng/mL) were constructed by plotting area under the curve (AUC) for each ceramide calibration standard d18:1/C16:0, d18:1/C18:0, d18:1/C20:0, d18:1/C22:0, and d18:1/C24:0 (Avanti polar lipids, Alabaster, AL, USA). Correlation coefficients for standard curves were >

0.999. Ceramide concentrations were calculated by fitting the identified ceramide species to these standard curves based on acyl chain length. Instrument control and data acquisition were performed by using Analyst (version 1.4.2, SCIEX Inc. Thornhill, Ontario, Canada) and data analysis were completed using MultiQuant software (version 2.0, SCIEX, Thornhill, ON, Canada).

Reproducibility of the LC-MS/MS method for ceramide analyses

The reproducibility of the current LC-MS/MS method for ceramide analysis was initially evaluated based on the several injections (n=15) of quality control samples and coefficient of variation (CoV) was 6.18% suggesting the suitability of the method (Supplementary Table 1). Moreover, the median of the average intra-run CoVs were within (±20%) across the entire cohort which indicates good reproducibility of the current LC-MS/MS method and instrument conditions (Supplementary Figure 2).

SUPPLEMENTARY REFERENCES

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Supplementary Table 1. Reproducibility of the current LC-MS/MS method for ceramide analyses were initially evaluated based on the multiple injections (n=15) of quality control sample at a known concentration

Sample Name	**Peak Area**
QC-Injection 1	2.52E+07
QC-Injection 2	2.92E+07
QC-Injection 3	2.51E+07
QC-Injection 4	2.31E+07
QC-Injection 5	2.21E+07
QC-Injection 6	2.50E+07
QC-Injection 7	2.43E+07
QC-Injection 8	2.37E+07
QC-Injection 9	2.44E+07
QC-Injection 10	2.32E+07
QC-Injection 11	2.40E+07
QC-Injection 12	2.42E+07
QC-Injection 13	2.53E+07
QC-Injection 14	2.42E+07
QC-Injection 15	2.48E+07
Mean	2.45E+07
SD	1.52E+06
CoV (%)	6.18%

QC: quality control; SD: standard deviation; CoV: coefficient of variation

Supplementary Table 2. Association of log-transformed lipid concentrations with age in HC

Lipid	Beta ± SE[a]	P-value[a]
Cer16:0	-0.001 ± 0.003	0.72
Cer18:0	0.004 ± 0.004	0.22
Cer20:0	0.004 ± 0.003	0.11
Cer22:0	0.006 ± 0.002	**0.011**
Cer24:0	0.001 ± 0.002	0.73
Cer26:0	0.007 ± 0.005	0.14
Cer18:1	0.002 ± 0.003	0.58
Cer20:1	-0.0004 ± 0.004	0.92
Cer22:1	0.004 ± 0.004	0.32
Cer24:1	0.006 ± 0.003	**0.043**
Cer26:1	0.003 ± 0.004	0.48
Hex-Cer16:1	0.012 ± 0.01	0.22
Hex-Cer18:1	-0.002 ± 0.003	0.51
Hex-Cer20:1	0.007 ± 0.002	**0.001**
Hex-Cer22:1	-0.002 ± 0.003	0.46
Hex-Cer24:1	-0.004 ± 0.003	0.28
Hex-Cer26:1	0.001 ± 0.006	0.91
Hex-Cer16:0	-0.007 ± 0.003	**0.014**
Hex-Cer18:0	-0.01 ± 0.008	0.2
Hex-Cer20:0	-0.002 ± 0.003	0.47
Hex-Cer22:0	-0.002 ± 0.003	0.56
Hex-Cer24:0	0.002 ± 0.003	0.41
Hex-Cer26:0	-0.005 ± 0.006	0.35
Lac-Cer16:1	-0.004 ± 0.002	0.09
Lac-Cer18:1	-0.003 ± 0.003	0.21
Lac-Cer20:1	0.006 ± 0.002	**0.003**
Lac-Cer22:1	0.0005 ± 0.004	0.9
Lac-Cer24:1	-0.008 ± 0.004	**0.04**
Lac-Cer26:1	-0.007 ± 0.003	**0.036**
Lac-Cer16:0	-0.009 ± 0.003	**<0.001**
Lac-Cer20:0	0.003 ± 0.002	0.12
Lac-Cer22:0	-0.001 ± 0.004	0.79
Lac-Cer24:0	0.0004 ± 0.003	0.89
DH-Cer18:0	0.002 ± 0.009	0.81
DH-Cer20:0	-0.001 ± 0.006	0.92
DH-Cer22:0	0.002 ± 0.003	0.5
DH-Cer24:0	0.002 ± 0.004	0.69
DH-Cer26:0	0.009 ± 0.004	0.06
DH-HexCer16:0	-0.003 ± 0.004	0.47
DH-HexCer18:0	-0.0003 ± 0.003	0.92
DH-HexCer20:0	0.0001 ± 0.005	0.99
DH-HexCer22:0	-0.001 ± 0.003	0.6
DH-HexCer24:0	0.002 ± 0.003	0.54
DH-HexCer26:0	0.001 ± 0.007	0.83
DH-LacCer16:0	-0.008 ± 0.003	**0.004**

[a] Univariable linear regression

HC: healthy controls; SE: standard error

Supplementary Table 3. Cross-sectional association of log-transformed lipid concentrations with EDSS

Lipid	Beta ± SE (unadjusted)[a]	P-value (unadjusted)[a]	Beta ± SE (adjusted)[b]	P-value (adjusted)[b]
Cer16:0	1.372 ± 0.424	**0.001**	0.693 ± 0.397	*0.08*
Cer20:0	1.215 ± 0.384	**0.002**	0.692 ± 0.354	*0.05*
Cer20:1	0.31 ± 0.263	0.24	0.179 ± 0.236	0.45
Cer22:1	0.014 ± 0.298	0.96	0.032 ± 0.269	0.91
Cer26:1	0.452 ± 0.266	0.09	-0.051 ± 0.252	0.84
DH-Cer20:0	0.875 ± 0.234	**<0.001**	0.451 ± 0.221	**0.042**
DH-Cer24:0	0.41 ± 0.334	0.22	-0.104 ± 0.307	0.74
Hex-Cer16:1	-0.498 ± 0.174	**0.005**	-0.242 ± 0.161	0.14
Hex-Cer20:1	-0.033 ± 0.61	0.96	0.05 ± 0.545	0.93
Hex-Cer22:1	-0.024 ± 0.34	0.94	0.143 ± 0.304	0.64
Hex-Cer24:1	0.243 ± 0.323	0.45	0.323 ± 0.288	0.26
Hex-Cer16:0	0.605 ± 0.332	0.07	0.56 ± 0.304	0.07
Hex-Cer18:0	0.496 ± 0.163	**0.003**	0.243 ± 0.151	0.11
Hex-Cer20:0	0.676 ± 0.261	**0.01**	0.374 ± 0.239	0.12
Hex-Cer22:0	0.29 ± 0.372	0.44	0.234 ± 0.333	0.48
Hex-Cer26:0	0.247 ± 0.254	0.33	-0.029 ± 0.231	0.9
DH-HexCer22:0	0.246 ± 0.37	0.51	0.254 ± 0.332	0.44
DH-HexCer26:0	-0.322 ± 0.175	0.07	-0.184 ± 0.159	0.25
Lac-Cer16:1	0.714 ± 0.442	0.11	0.446 ± 0.399	0.26
Lac-Cer20:1	0.584 ± 0.534	0.28	0.684 ± 0.476	0.15
Lac-Cer24:1	0.81 ± 0.331	**0.015**	0.625 ± 0.3	**0.039**
Lac-Cer16:0	0.885 ± 0.448	**0.049**	0.664 ± 0.404	0.1
Lac-Cer22:0	0.736 ± 0.43	0.09	0.317 ± 0.389	0.42

[a] Univariable linear regression

[b] Multivariable linear regression including age, sex, race as covariates

EDSS: Expanded Disability Status Scale; SE: Standard Error; MS: multiple sclerosis

Supplementary Table 4. Cross-sectional association of log-transformed lipid concentrations with ARMSS

Lipid	Beta ± SE (unadjusted)[a]	P-value (unadjusted)[a]	Beta ± SE (adjusted)[b]	P-value (adjusted)[b]
Cer16:0	0.762 ± 0.515	0.14	0.65 ± 0.518	0.21
Cer20:0	0.693 ± 0.466	0.14	0.711 ± 0.463	0.13
Cer20:1	0.168 ± 0.315	0.59	0.227 ± 0.314	0.47
Cer22:1	0.012 ± 0.357	0.97	0.145 ± 0.359	0.69
Cer26:1	-0.247 ± 0.319	0.44	-0.149 ± 0.321	0.64
DH-Cer20:0	0.425 ± 0.286	0.14	0.398 ± 0.285	0.16
DH-Cer24:0	-0.234 ± 0.4	0.56	-0.211 ± 0.399	0.6
Hex-Cer16:1	-0.259 ± 0.211	0.22	-0.214 ± 0.212	0.31
Hex-Cer20:1	0.41 ± 0.729	0.57	0.455 ± 0.725	0.53
Hex-Cer22:1	0.244 ± 0.406	0.55	0.239 ± 0.404	0.55
Hex-Cer24:1	0.408 ± 0.386	0.29	0.436 ± 0.384	0.26
Hex-Cer16:0	0.784 ± 0.397	**0.049**	0.633 ± 0.405	0.12
Hex-Cer18:0	0.264 ± 0.198	0.18	0.231 ± 0.197	0.24
Hex-Cer20:0	0.425 ± 0.316	0.18	0.381 ± 0.315	0.23
Hex-Cer22:0	0.426 ± 0.445	0.34	0.338 ± 0.444	0.45
Hex-Cer26:0	-0.090 ± 0.305	0.77	-0.095 ± 0.303	0.75
DH-HexCer22:0	0.456 ± 0.442	0.3	0.366 ± 0.442	0.41
DH-HexCer26:0	-0.272 ± 0.21	0.2	-0.216 ± 0.211	0.31
Lac-Cer16:1	0.376 ± 0.531	0.48	0.477 ± 0.529	0.37
Lac-Cer20:1	1.384 ± 0.634	**0.03**	1.356 ± 0.63	**0.033**
Lac-Cer24:1	0.792 ± 0.398	**0.048**	0.769 ± 0.4	*0.06*
Lac-Cer16:0	0.773 ± 0.538	0.15	0.647 ± 0.538	0.23
Lac-Cer22:0	0.371 ± 0.517	0.47	0.335 ± 0.515	0.52

[a] Univariable linear regression
[b] Multivariable linear regression including sex, race as covariates

ARMSS: Age-related Multiple Sclerosis Severity Score; SE: Standard Error; MS: multiple sclerosis

Supplementary Table 5. Association of baseline lipid concentration with odds of EDSS worsening

Lipid	OR (95% CI) (unadjusted)[a]	P-value (unadjusted)[a]	OR (95% CI) (adjusted)[b]	P-value (adjusted)[b]
Cer16:0	3.84 (1.41 to 10.43)	**0.008**	2.97 (1.03 to 8.59)	**0.044**
Cer20:0	2.09 (0.86 to 5.12)	0.11	2.03 (0.78 to 5.26)	0.15
Cer20:1	1.54 (0.86 to 2.76)	0.14	1.6 (0.86 to 2.96)	0.14
Cer22:1	1.68 (0.83 to 3.36)	0.15	2.05 (0.97 to 4.35)	0.06
Cer26:1	1.63 (0.9 to 2.98)	0.11	1.99 (0.99 to 3.97)	0.05
DH-Cer20:0	1.75 (1 to 3.05)	*0.05*	1.61 (0.89 to 2.9)	0.11
DH-Cer24:0	1.72 (0.8 to 3.68)	0.16	1.75 (0.77 to 3.96)	0.18
Hex-Cer16:1	0.72 (0.49 to 1.07)	0.11	0.79 (0.52 to 1.21)	0.28
Hex-Cer20:1	0.72 (0.2 to 2.59)	0.62	0.65 (0.17 to 2.5)	0.53
Hex-Cer22:1	1.78 (0.84 to 3.79)	0.13	1.83 (0.82 to 4.09)	0.14
Hex-Cer24:1	1.52 (0.73 to 3.15)	0.26	1.65 (0.77 to 3.55)	0.2
Hex-Cer16:0	2.33 (1.1 to 4.91)	**0.027**	1.89 (0.87 to 4.13)	0.11
Hex-Cer18:0	1.29 (0.9 to 1.85)	0.16	1.21 (0.82 to 1.77)	0.33
Hex-Cer20:0	1.67 (0.95 to 2.96)	0.08	1.54 (0.84 to 2.83)	0.17
Hex-Cer22:0	2.3 (0.98 to 5.37)	*0.05*	2.12 (0.86 to 5.23)	0.1
Hex-Cer26:0	1.64 (0.90 to 2.97)	0.11	1.63 (0.86 to 3.1)	0.13
DH-HexCer22:0	2.12 (0.93 to 4.86)	0.08	1.96 (0.81 to 4.72)	0.13
DH-HexCer26:0	0.72 (0.48 to 1.07)	0.11	0.78 (0.51 to 1.19)	0.25
Lac-Cer16:1	1.35 (0.51 to 3.57)	0.55	1.47 (0.52 to 4.17)	0.47
Lac-Cer20:1	0.52 (0.17 to 1.63)	0.26	0.44 (0.13 to 1.48)	0.18
Lac-Cer24:1	1.61 (0.78 to 3.33)	0.2	1.59 (0.73 to 3.43)	0.24
Lac-Cer16:0	2.06 (0.79 to 5.4)	0.14	1.8 (0.66 to 4.91)	0.25
Lac-Cer22:0	2.16 (0.81 to 5.79)	0.13	2.1 (0.75 to 5.9)	0.16

[a] Univariable logistic regression

[b] Multivariable logistic regression including age, sex, race as covariates

EDSS: Expanded Disability Status Scale; OR: odds ratio; CI: Confidence Interval; MS: Multiple Sclerosis

Supplementary Table 6. Demographic characteristics of the longitudinal OCT-lipidomics cohort

Characteristic	Value
Subjects (eyes)	180 (345)
Age (years), mean (SD)	44.0 (11.9)
Female Sex, n (%)	131 (73%)
Race, n (%)	
Caucasian American	144 (80%)
African American	25 (14%)
Other	11 (6%)
MS subtype, n (%)	
RRMS	117 (65%)
PMS	63 (35%)
DMT at baseline[a]	
None	33 (18%)
High potency	49 (27%)
Intermediate potency	10 (6%)
Low potency	83 (46%)
Other	5 (3%)
EDSS at baseline, median (IQR)	2.5 (1.5-4)
Disease duration (years), median (IQR)	9 (6-14.5)
Follow-up (years), median (IQR)	5.4 (3.5-6.6)
Eyes with a history of optic neuritis, n (%)	112 (32%)

[a] Glatiramer acetate, interferon-beta, and teriflunomide were classified as low-potency DMTs, dimethyl fumarate and fingolimod as intermediate-potency DMTs, and natalizumab, ocrelizumab, rituximab, alemtuzumab, and daclizumab as high-potency DMTs.

OCT: optical coherence tomography; MS: Multiple Sclerosis; RRMS: Relapsing-Remitting MS; PMS: Progressive MS; SD: Standard Deviation; DMT: Disease Modifying Treatment; EDSS: Expanded Disability Status Scale; IQR: Interquartile range

Supplementary Table 7. Association of baseline log-transformed lipid concentrations with rates of GCIPL thinning (µm/year)

Lipid	Beta ± SE (unadjusted)[a]	P-value (unadjusted)[a]	P-value (adjusted)[b]
Cer16:0	-0.056 ± 0.054	0.3	0.47
Cer20:0	-0.076 ± 0.054	0.16	0.17
Cer20:1	-0.034 ± 0.034	0.31	0.37
Cer22:1	-0.064 ± 0.043	0.14	0.21
Cer26:1	0 ± 0.037	>0.99	0.56
DH-Cer20:0	-0.045 ± 0.034	0.18	0.33
DH-Cer24:0	-0.045 ± 0.05	0.37	0.62
Hex-Cer16:1	0.03 ± 0.025	0.23	0.29
Hex-Cer20:1	-0.084 ± 0.075	0.26	0.30
Hex-Cer22:1	-0.065 ± 0.042	0.12	0.10
Hex-Cer24:1	-0.116 ± 0.046	**0.012**	**0.010**
Hex-Cer16:0	-0.011 ± 0.042	0.8	0.76
Hex-Cer18:0	-0.014 ± 0.021	0.5	0.77
Hex-Cer20:0	-0.045 ± 0.034	0.19	0.23
Hex-Cer22:0	-0.138 ± 0.053	**0.01**	**0.009**
Hex-Cer26:0	-0.032 ± 0.038	0.4	0.74
DH-HexCer22:0	-0.158 ± 0.053	**0.003**	**0.002**
DH-HexCer26:0	-0.015 ± 0.025	0.56	0.48
Lac-Cer16:1	-0.001 ± 0.057	0.98	0.61
Lac-Cer20:1	-0.051 ± 0.068	0.46	0.70
Lac-Cer24:1	-0.042 ± 0.045	0.36	0.46
Lac-Cer16:0	0.011 ± 0.059	0.85	0.72
Lac-Cer22:0	-0.064 ± 0.055	0.25	0.42

[a] Univariable mixed-effects linear regression with subject-specific and eye-specific random intercepts and random slopes in time

[b] Multivariable mixed effects linear regression including age, sex, race, MS subtype, ON history and their respective interactions with follow-up time

GCIPL: Ganglion Cell + Inner Plexiform Layer; SE: Standard Error; MS: Multiple Sclerosis; ON: optic neuritis

Supplementary Table 8. Association of baseline log-transformed lipid concentrations with rates of pRNFL thinning (µm/year)

Lipid	Beta ± SE (unadjusted)[a]	P-value (unadjusted)[a]	P-value (adjusted)[b]
Cer16:0	-0.041 ± 0.104	0.69	0.26
Cer20:0	-0.137 ± 0.115	0.23	**0.019**
Cer20:1	-0.1 ± 0.071	0.16	**0.035**
Cer22:1	-0.126 ± 0.093	0.17	**0.017**
Cer26:1	0.104 ± 0.075	0.17	0.94
DH-Cer20:0	-0.051 ± 0.068	0.45	0.11
DH-Cer24:0	-0.064 ± 0.099	0.52	0.07
Hex-Cer16:1	0.002 ± 0.051	0.97	0.75
Hex-Cer20:1	0.133 ± 0.148	0.37	0.37
Hex-Cer22:1	-0.217 ± 0.089	**0.015**	**0.014**
Hex-Cer24:1	-0.14 ± 0.095	0.14	0.08
Hex-Cer16:0	-0.214 ± 0.084	**0.011**	**0.025**
Hex-Cer18:0	-0.027 ± 0.043	0.54	0.31
Hex-Cer20:0	-0.088 ± 0.069	0.2	*0.05*
Hex-Cer22:0	-0.305 ± 0.107	**0.004**	**0.001**
Hex-Cer26:0	-0.113 ± 0.075	0.13	**0.039**
DH-HexCer22:0	-0.358 ± 0.106	**0.001**	**<0.001**
DH-HexCer26:0	-0.056 ± 0.052	0.28	0.45
Lac-Cer16:1	-0.05 ± 0.116	0.67	0.12
Lac-Cer20:1	0.067 ± 0.138	0.63	0.76
Lac-Cer24:1	-0.099 ± 0.091	0.28	0.09
Lac-Cer16:0	-0.149 ± 0.119	0.21	0.09
Lac-Cer22:0	-0.2 ± 0.113	0.08	**0.014**

[a] Univariable mixed-effects linear regression with subject-specific and eye-specific random intercepts and random slopes in time

[b] Multivariable mixed effects linear regression including age, sex, race, MS subtype, ON history and their respective interactions with follow-up time

pRNFL: Peripapillary Retinal Nerve Fiber Layer; SE: Standard Error; MS: Multiple Sclerosis; ON: optic neuritis

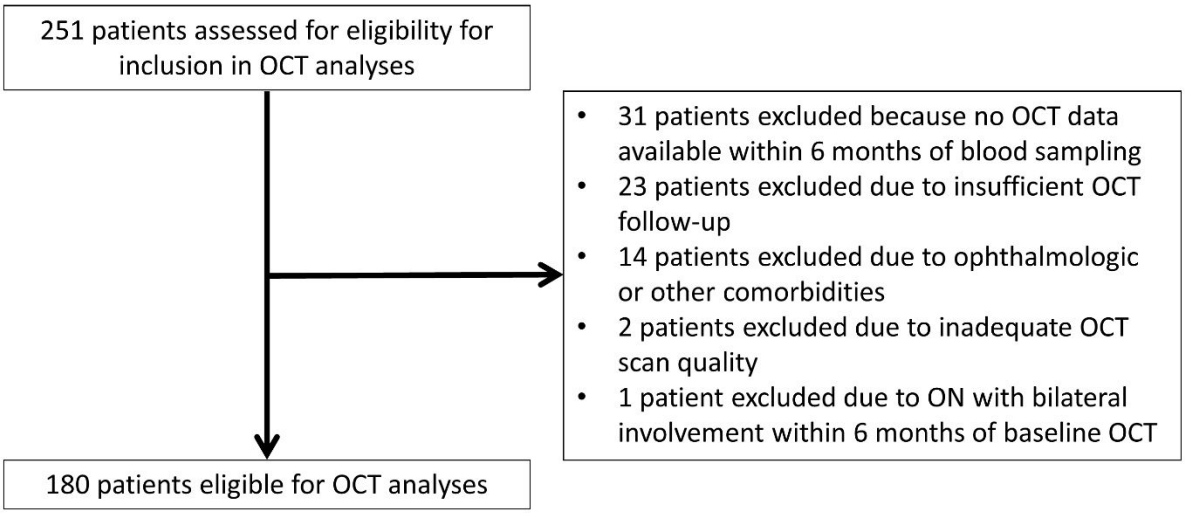
Supplementary Table 9. Cross-sectional association of log-transformed lipid concentrations with sNfL

Lipid	Beta ± SE (adjusted)[a]	P-value (adjusted)[a]
Cer16:0	0.022 ± 0.039	0.57
Cer20:0	0.05 ± 0.04	0.22
Cer20:1	-0.044 ± 0.061	0.48
Cer22:1	-0.004 ± 0.054	0.94
Cer26:1	-0.047 ± 0.057	0.42
DH-Cer20:0	0.083 ± 0.064	0.2
DH-Cer24:0	0.009 ± 0.046	0.85
Hex-Cer16:1	-0.106 ± 0.084	0.21
Hex-Cer20:1	-0.011 ± 0.027	0.7
Hex-Cer22:1	-0.0003 ± 0.046	>0.99
Hex-Cer24:1	0.059 ± 0.049	0.23
Hex-Cer16:0	-0.0001 ± 0.048	>0.99
Hex-Cer18:0	0.078 ± 0.09	0.38
Hex-Cer20:0	0.028 ± 0.055	0.61
Hex-Cer22:0	0.086 ± 0.042	**0.043**
Hex-Cer26:0	0.011 ± 0.06	0.86
DH-HexCer22:0	0.073 ± 0.042	0.08
DH-HexCer26:0	-0.038 ± 0.09	0.68
Lac-Cer16:1	0.039 ± 0.036	0.29
Lac-Cer20:1	0.011 ± 0.03	0.73
Lac-Cer24:1	0.072 ± 0.047	0.12
Lac-Cer16:0	0.038 ± 0.035	0.28
Lac-Cer22:0	0.037 ± 0.036	0.31

[a] Multivariable linear regression including age as covariate

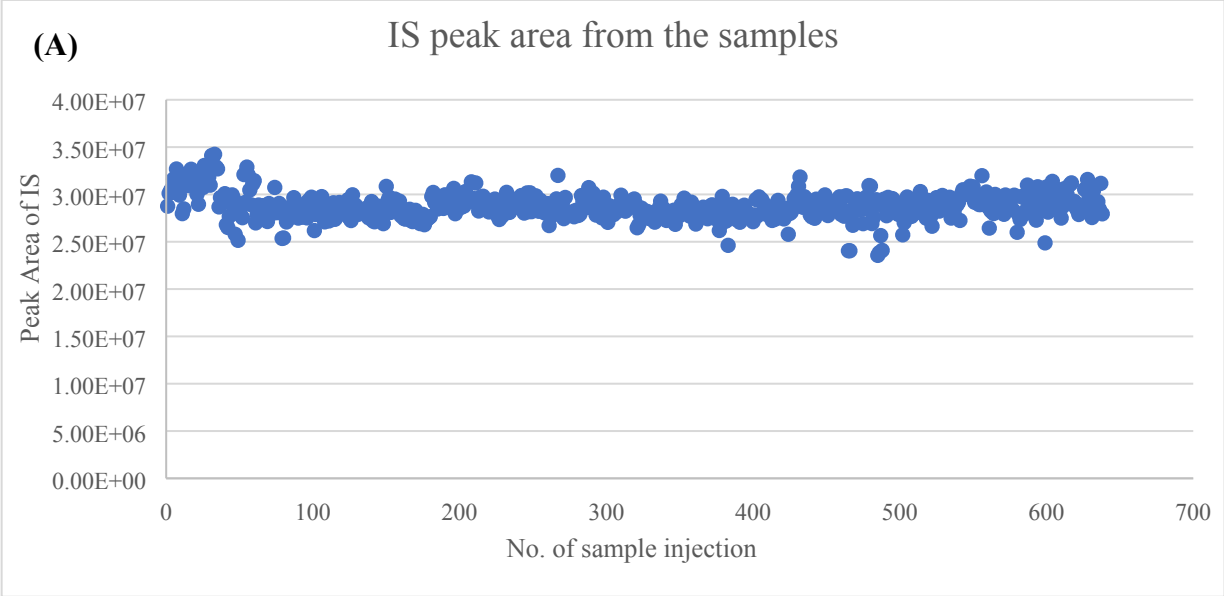
sNfL: serum neurofilament light chain ; SE: Standard Error; MS: multiple sclerosis

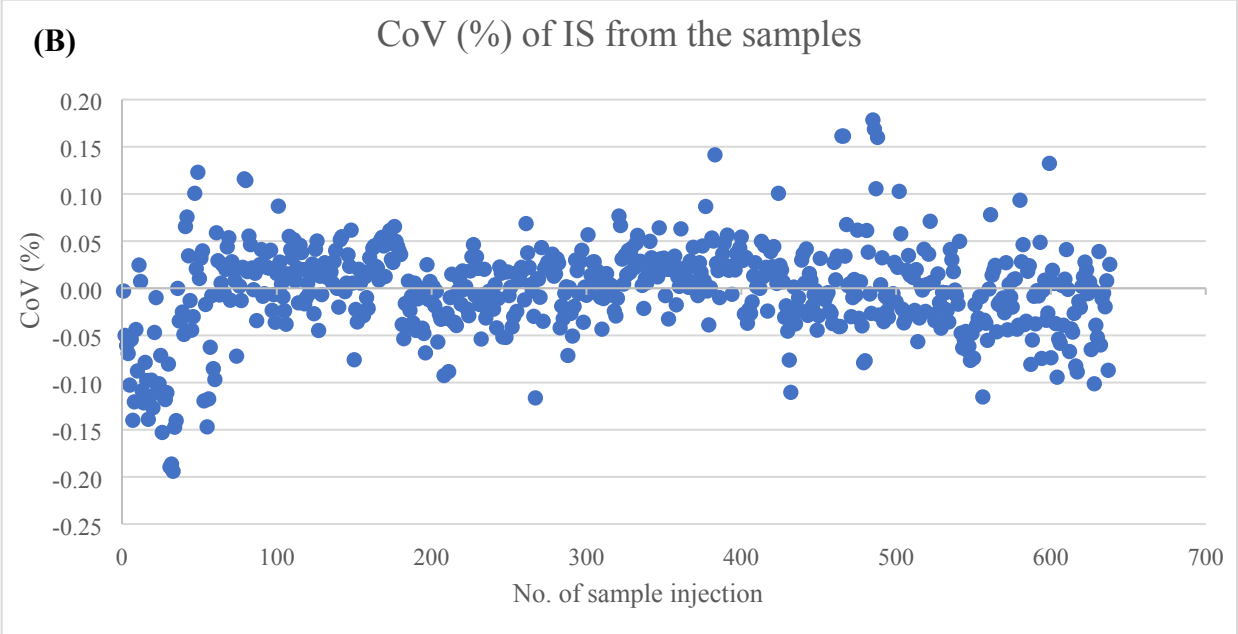
Supplementary Figure 1. Flowchart for the longitudinal OCT-lipidomics cohort



OCT: Optical Coherence Tomography; ON: optic neuritis

Supplementary Figure 2. Reproducibility of the internal standards spiked in each sample using current LC-MS/MS method for ceramide analyses (A) peak area of internal standard from each sample injection (B) CoV (%) of peak areas of internal standard from each sample injection. Duplicate injection (n=2) was performed for each sample. Data are showing from a total of 638 injections and the median of the average intra-run % CoV were within (±20%) across the entire cohort indicating the good reproducibility of the current LC-MS/MS method and instrument condition.





IS: internal standard; CoV: coefficient of variation