

Supplementary material

Supplementary table 1. Genetics cohort population characteristics

Characteristics	Subcohorts with measures of disease activity			
	Total (N=604)	Disability (N=563)	Relapse (N=511)	MRI (N=208)
Gender: Female/Male (% Female)	409/195 (68%)	383/180 (68%)	359/152 (70%)	149/59 (72%)
Disease Course: bout onset (BO) /PP/unknown (% BO)	526/63/15 (87%)	486/62/15 (86%)	511/0/0 (100%)	203/2/3 (98%)
Age at onset (yrs): median (IQR)	32 (25-41)	32 (25-41)	31 (24-39)	29 (23-38)
Time from onset to most recent EDSS (yrs): median (IQR)	—	12 (6-18)	—	—
EDSS most recent: median (IQR)	—	2 (1-5)	—	—
ARMSS most recent: median (IQR)	—	3.22 (1.28-6.00)	—	—
Time before treatment from onset (yrs): median (IQR)	—	—	2 (0-5)	—
Annualized relapse rate before treatment: median (IQR)	—	—	0.67 (0.25-1.6)	—
Total follow-up time from onset (yrs): median (IQR)	—	—	13 (7-20)	—
Annualized relapse rate, total: median (IQR)	—	—	0.29 (0.17-0.46)	—
Time from onset to MRI (yrs): median (IQR)	—	—	—	8 (4-14)

ARMSS = Age-related Multiple Sclerosis Severity Score, yrs= years, BO = bout onset, EDSS = Expanded Disability Status Scale, IQR=Interquartile Range, PP = primary progressive

Supplementary table 2. Biomarker measurement quality control.

	N measured	Inter-plate CV (%)	Within-plate mean CV (%)	N CV>25%	N < or > detection range	N included (%)
CSF N = 143						
NfL	143	12.30%	13.46%	15	1 < and 6 >	122 (85.3%)
CHIT1	143	6.60%	5.91%	5	6 < and 1 >	129 (90.2%)
CHI3L1	143	17.70%	3.38%	0	1 < and 1 >	141 (98.6%)
sTREM2	140	10.1%	7.08%	7	20 <	113 (80.7%)
Serum N = 78						
CHIT1	78	7.80%	4.5%	0	0	78 (100%)

CV = coefficient of variation.

Supplementary table 3. Overview of MRI scanning protocols.

Protocol	Number of scans (%)	Scanner	Sequence	Flip Angle (°)	Echo Time (ms)	Repetition Time (ms)	Pixel Spacing (mm x mm)	Slice Thickness (mm)
A	9 (11%)	Achieva	MTR	15	7.99 or 8.0	68.62	1.00x1.00	3
			3D-TFE	8	4.6	9.55-9.63	0.87x0.87 or 0.98x0.98	1.2
			3D-FLAIR	90	339.29-406.27	4800	1.04x1.04	1.12
B	4 (5%)	Achieva dstream	MTR	10	4.59	67.81	1.00x1.00	3
			3D-TFE	8	4.6	9.53-9.61	0.98x0.98	1.2
			3D-FLAIR	90	331.86-366.83	4800	1.04x1.04	1.12
C	17 (22%)	Ingenia	MTR	10	4.59 or 4.6	70.66	1.14x1.14	3
			3D-TFE	8	4.6 or 4.61	9.59-9.77	0.98x0.98	1.2
			3D-FLAIR	90	343.69-404.31	4800	1.04x1.04	1.12 or 1.2
D	36 (46%)	Ingenia	MTR	10	4.59 or 4.6	67.75	1.00x1.00	3
			3D-TFE	8	4.6 or 4.61	9.57-9.8	0.98x0.98, 0.87x0.87 or 0.78x0.78	1.2
			3D-FLAIR	90	323.75-407.83	4800	1.04x1.04	1.12
E	4 (5%)	Ingenia	MTR	10	4.59	70.66	1.14x1.14	3
			3D-TFE	8	4.6 or 4.61	9.63-9.74	0.98x0.98	1.2
			3D-FLAIR	90	345.38-392.99	4800	1.04x1.04	1.12 or 1.2
F	9 (11%)	Intera	MTR	10	4.59	82.58	1.00x1.00	3
			3D-TFE	8	4.6	9.58-9.65	0.98x0.98	1.2
			3D-FLAIR	90	344.47-392.6	4800	1.04x1.04	1.2

Number of scans is given for N=79 patients in the CSF analysis cohort.

Supplementary table 4. Descriptive correlation between potential CSF markers in this study and clinically used CSF characteristics

vs	NfL		CHIT1		CHI3L1		sTREM2	
	β	p-value	β	p-value	β	p-value	β	p-value
IgG index	0.04	0.41	0.09	0.011	0.25	0.0016	0.13	0.10
Albumin ratio	0.05	0.79	0.37	0.037	0.74	0.029	0.15	0.63
OCB positive	0.01	0.84	-0.06	0.25	-0.25	0.037	-0.07	0.49
OCB count	2.88	0.03	2.79	0.023	9.75	9.16E-05	4.1	0.07
WBC	-0.02	0.90	6.22	0.33	13.2	0.30	-0.10	0.99

IgG = immunoglobulin G, OCB = oligoclonal bands, WBC = white blood cell count.

Linear regression analysis after applying a log10 transformation to biomarker measurements and an inverse rank normal transformation for WBC count. Age at LP, gender and rs4950928 for CHI3L1 and rs150192398 genotype for CHIT1, were used as covariates. Uncorrected p-values $p < 0.05$ are indicated in bold.

Supplementary table 5. Multiple linear regression of baseline CSF biomarkers with clinical and MRI parameters at follow-up.

NEDA parameter		Variable (outcome)	NfL		CHIT1		CHI3L1	
			β	p	β	p	β	p
Disability		ARMSS (I)	0.10	0.66	0.69	0.015	-0.37	0.58
		MSSS (II)	0.28	0.23	0.64	0.027	-0.82	0.23
		EDSS (II)	0.13	0.80	2.00	0.003	-0.76	0.63
Relapse		ARR, untreated (I)	0.37	0.13	0.42	0.15	0.42	0.53
		ARR, all (II)	0.25	0.34	0.78	0.014	0.57	0.42
MRI	volumetric	GM vol (% tot. Brain vol) (I)	0.14	0.61	-0.55	0.09	0.96	0.12
		WM vol (% tot. Brain vol) (I)	-0.29	0.43	-0.77	0.09	0.90	0.29
		Lesion vol (mm ³) (I)	0.26	0.38	1.07	0.005	-0.94	0.48
	MTR	Median MTR NAGM (I)	-0.15	0.68	-0.37	0.40	0.58	0.48
		Peak Height MTR NAGM (I)	-0.49	0.11	-0.25	0.49	0.09	0.89
		Median MTR NAWM (I)	-0.06	0.86	-0.58	0.20	0.62	0.47
		Peak Height MTR NAWM (I)	-0.05	0.88	-0.17	0.69	0.22	0.80
		Median MTR Lesion (I)	0.18	0.63	-0.74	0.10	0.64	0.45
		Peak Height MTR Lesion (I)	0.23	0.51	-0.75	0.08	0.42	0.61

Multiple linear regression was performed for markers which showed a nominally significant association with at least one outcome. A logarithmic transformation with base 10 for biomarker measurements, and an inverse rank normal transformation for outcome variables that are not normally distributed were applied. For MRI correlations, age at MRI, gender (for GM volume only) and MRI protocol were used as covariates. For CHI3L1, age, gender and rs4950928 genotype, and for CHIT1 rs150192398, were used as covariates. Primary (I) and secondary (II) outcomes are indicated. Nominally significant p-values (uncorrected $P < 0.05$) are in bold.

ARMSS = Age Related Multiple Sclerosis Severity Score; MTR = magnetization transfer ratio, NAGM = normal appearing grey matter; NAWM = normal appearing white matter; GM = Grey matter; WM = White matter; AAR=annualized relapse rate

Supplementary table 6. Association of functional genetic variation in *CHIT1* and *CHI3L1* genes with primary outcomes.

Variable	CHIT1				CHI3L1			
	SNP	β	p-value	N	SNP	β	p-value	N
ARMSS	rs150192398	0.05	0.50	563	rs4950928	0.07	0.37	563
ARR, untreated	rs150192398	0.04	0.64	511	rs4950928	-0.04	0.60	511
GM vol (% tot. Brain vol)	rs150192398	-0.20	0.42	208	rs4950928	0.09	0.71	208
WM vol (% tot. Brain vol)	rs150192398	0.21	0.43	208	rs4950928	0.09	0.75	208
Lesion volume (mm ³)	rs150192398	0.32	0.35	208	rs4950928	-0.24	0.48	208
Median MTR NAGM	rs150192398	0.09	0.72	208	rs4950928	0.02	0.95	208
Peak Height MTR NAGM	rs150192398	-0.06	0.84	208	rs4950928	0.17	0.57	208
Median MTR NAWM	rs150192398	0.16	0.49	208	rs4950928	-0.11	0.65	208
Peak Height MTR NAWM	rs150192398	-0.21	0.48	208	rs4950928	0.30	0.31	208
Median MTR Lesion	rs150192398	0.03	0.90	208	rs4950928	-0.02	0.95	208
Peak Height MTR Lesion	rs150192398	-0.37	0.16	208	rs4950928	0.46	0.09	208

β (for major allele) and p-value from simple linear regression analysis. Age at MTR and MRI protocol were used as covariates for MRI outcomes. ARMSS = Age Related Multiple Sclerosis Severity Score, MTR = Magnetization transfer ratio, ARR=Annualized relapse rate