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Original research

Plasma CHI3L1 associates with brain volume loss and glial activation in multiple sclerosis

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ABSTRACT

Background Multiple sclerosis (MS) progression independent of relapses is driven by brain innate immune cell activation. The aim of this study was to evaluate the association between chitinase-3-like protein 1 (CHI3L1), expressed in brain by astrocytes and microglia, measured from blood and smouldering inflammation measured using 18 kDa translocator protein (TSPO) positron emission tomography (PET) in patients with MS.

Methods The study cohort included 55 patients with MS (25 progressive MS (PMS) and 30 relapsing remitting MS (RRMS)) and 17 healthy controls (HC). CHI3L1 was measured with commercial ELISA from plasma samples. A subcohort (44 MS and 9 HC) underwent TSPO-PET to assess [^{11}C]PK11195 distribution volume ratio (DVR) and MRI concurrent to blood sampling. These imaging outcomes were used in respective correlation and linear regression analyses.

Results CHI3L1 concentration in plasma was higher in PMS (23.5 ng/mL) compared with HC (16.8 ng/mL, p=0.0055) and RRMS (19.3 ng/mL, p=0.049). CHI3L1 associated with brain [^{11}C]PK11195 DVR in all MS (standardised estimate 0.89, 95% CI 0.23 to 1.55, p=0.010) and in PMS (Spearman correlation ρ=0.58, 95% CI 0.058 to 0.86, p=0.032). Additionally, CHI3L1 was associated with smaller brain volume in both MS (−0.75, −1.38 to −0.11, p=0.023) and PMS (ρ=−0.56, −0.83 to −0.095, p=0.021). Furthermore, CHI3L1 was associated with Expanded Disability Status Scale (0.70, 0.12 to 1.28, p=0.019) and age (0.93, 0.37 to 1.48, p=0.002) among all patients with MS.

Conclusions Association of CHI3L1 with glial activation and brain volume loss identifies plasma CHI3L1 as a promising biomarker for smouldering inflammation and MS progression-related pathology.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Chitinase-3-like protein 1 (CHI3L1) level in the CSF is higher in progressive MS compared with relapsing remitting MS and healthy controls. The results regarding peripheral blood CHI3L1 in MS vary considerably between different studies.

WHAT THIS STUDY ADDS

⇒ Our study shows that plasma CHI3L1 is increased in progressive MS and associates both with an increased TSPO-PET signal, an imaging biomarker for glial activity known to associate with MS progression, and with brain atrophy.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our study suggests that blood CHI3L1 could be used as a biomarker for MS progression-related pathology.

INTRODUCTION

Microglia normally protect the central nervous system (CNS) against insults, and in multiple sclerosis (MS), they provide help in repairing myelin damage by clearing myelin debris. However, their constant and prolonged activation associated with chronic neuroinflammation leads to the production of pro-inflammatory cytokines and free radicals and alterations in iron homeostasis which may induce damage to neurons and oligodendrocytes.[1] Microglial activation and increased density can be imaged in vivo using 18 kDa translocator protein positron emission tomography (TSPO-PET).[2] Increased glial activation measured with TSPO-PET associates with higher Expanded Disability Status Scale (EDSS) and can be used as a prognostic marker for MS worsening independent of relapses.[3–5] Currently, there are no blood biomarkers for microglial activation as the two most established blood biomarkers in the MS field are glial fibrillary acidic protein (GFAP)[6] indicating astrocytic injury and activation, and neurofilament light chain (NfL)[7] signifying axonal damage.

Chitinase-3-like protein 1 (CHI3L1) lacks the enzymatic activity of chitinases and belongs to chitinase-like proteins in the glycoside hydrolase family 18. It is secreted by several cells such as macrophages, endothelial cells and smooth muscle cells, and it regulates various vital functions through a number of signalling pathways.[8] Using histopathological staining and single-cell RNA-sequencing, it has been found that in the brain, CHI3L1 is most prominently expressed in astrocytes and microglia, particularly in activated cells[9] and MS-associated microglia[10] located primarily in active and chronic active lesions but also in the normal appearing

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Table 1 Demographics, clinical characteristics and biomarker concentrations of patients with MS and HCs

	HC	MS	P value*	PMS	RRMS	P value*
n	17	55		25	30	
Female n (%)	11 (65)	37 (67)	>0.99	16 (64)	21 (70)	0.77
Age	44.6 (9.30)	47.3 (9.60)	0.31	53.3 (9.30)	42.2 (6.47)	**<0.0001**
BMI	24.9 (23.0–27.4)	26.8 (22.1–33.2)	0.27	22.5 (20.2–28.2)	28.5 (25.5–34.8)	**0.0039**
Disease duration†		11.8 (2.97–17.4)		16.8 (9.42–19.2)	6.93 (1.42–13.4)	**0.0006**
EDSS		3 (2.00–5.00)		5.5 (3.5–6.5)	2 (1–2.625)	**<0.0001**
MSSS		3.79 (1.34–6.50)		4.63 (3.52–7.44)	1.70 (0.69–5.16)	**0.0005**
ARR		0.3 (0.2–0.64)		0.3 (0.14–0.54)	0.3 (0.2–0.82)	0.37
Gd+lesions n (%)‡		7 (16)		2 (9)	5 (25)	0.22
Relapse-sampling§		4.0 (1.0–8.7)		6.6 (1.3–10)	2.0 (0.57–8.1)	0.06
DMT n(%)		27(49)		13(52)	14(47)	0.79
CHI3L1 (ng/ml)	16.8 (16.2–24.5)	20.8 (16.6–36.2)	0.0675	23.5 (19.0–37.6)	19.3 (14.8–29.4)	**0.0488**
GFAP (pg/ml)¶	55.5 (43.7–85.5)	104.2 (70.5–136.5)	**0.0004**	132.4 (94.9–168.2)	77.0 (65.0–123.0)	**0.0049**
NfL (pg/ml)¶	8.20 (4.0–9.2)	10.2 (6.88–16.3)	**0.0098**	15.0 (9.80–22.4)	8.5 (6.5–13.7)	**0.0375**

Continuous variables are presented as median and quartiles (Q1–Q3) or mean and standard deviation (SD) unless specified otherwise.
*From comparison of HC and MS or PMS and RRMS with Fisher's exact test (categorical variables), two-tailed T-test (age) or Mann-Whitney U-test. Significant p values are bolded.
†From diagnosis to sampling.
‡Data were available from 43 patients with MS, 20 with RRMS and 23 with PMS.
§Time between last relapse and sampling (years).
¶Five missing values in HC and nine in PMS.
ARR, annualised relapse rate; BMI, body mass index; CHI3L1, chitinase-3-like protein 1; DMT, disease-modifying treatment; EDSS, expanded disability status scale; Gd, gadolinium; GFAP, glial fibrillary acidic protein; HC, healthy control; MS, multiple sclerosis; MSSS, multiple sclerosis severity score; NfL, neurofilament light chain; PMS, progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis.

white matter (NAWM).[9 11 12] The role of CHI3L1 in MS pathogenesis remains largely unknown, but mouse models have implicated that CHI3L1 participates in the inflammatory response by altering cytokine production.[13] Furthermore, it has been stated that CHI3L1 is cytotoxic to neurons in vitro[14] and impairs neurogenesis in vivo.[15] Several studies have found higher levels of CHI3L1 in progressive MS (PMS) compared with healthy controls (HCs) and relapsing remitting MS (RRMS) both in the cerebrospinal fluid (CSF)[16] and in the blood.[9 17 18] Furthermore, a large meta-analysis found that CSF CHI3L1 was associated with EDSS in primary progressive MS (PPMS).[16]

In order to get further insight into the potential value of blood CHI3L1 as a biomarker of MS progression-related glial activation, we evaluated the relationship between CHI3L1 plasma concentration and glial activation measured using TSPO-PET.

METHODS

Study subjects

Patients with MS were recruited from the outpatient clinic of the Division of Clinical Neurosciences at the University Hospital Turku, Turku, Finland during 2009–2022. All patients had been diagnosed according to the McDonald Criteria[19] and had a blood sample taken and EDSS assessed concurrently. The study cohort consisted of 55 patients with MS (n(SPMS)=21, n(PPMS)=4, n(RRMS)=30) and 17 age- and sex-matched HCs. Half of the patients with MS (49%) were using disease modifying treatment at the time of study onset (fingolimod (n=3), glatiramer acetate (n=2), natalizumab (n=6), rituximab (n=8), teriflunomide (n=8)). A proportion of study subjects (14 PMS, 30 RRMS and nine HCs) had TSPO-PET performed at Turku PET Centre within 6 months of blood sampling. This subcohort was included in the part of the study evaluating imaging and biomarker associations. The mean (range) time between blood sampling and PET in the subcohort was 15 (0–93) days for patients with MS and 45 (0–152) days for HCs.

Chitinase-3-like protein (CHI3L1), glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL) measurements

Blood was collected into Vacuette 10 mL K2EDTA and serum clot-activator tubes before noon. For serum, the blood was first allowed to coagulate for 30 min at RT. Then, both tubes were centrifuged (2000 g, 10 min, RT), and plasma and serum were frozen within 2 hours of sampling at −80 °C. Samples were stored in Auria Biobank (Turku, Finland).

Plasma CHI3L1 was measured in duplicates using quantitative sandwich enzyme immunoassay technique (ELISA). Human Chitinase 3-like 1/YKL-40 Quantikine ELISA-Kit (DC3L10, R&D Systems) was used following manufacturer's instructions with recommended dilution 1:50. Optical densities were measured using a HIDEX Sense microplate reader (Hidex Oy, Turku, Finland) at wavelengths 450 nm and 570 nm, and for the analyses, a subtracted value OD(450)-OD(570) was calculated to correct optical imperfections in the microplate. A standard curve was created using 4PL curve fit with Origin 2016. One plasma sample had slightly higher optical density than the highest standard and was determined to have the concentration of the highest standard (200 ng/mL). Mean intra-assay coefficients of variation for samples were 3% and 5% (plates 1 and 2, respectively) and for standards 2% and 3%.

Serum GFAP and NfL measurements were performed using single molecule array (SIMOA) and Neurology 2 plexB assay (Quanterix) in Basel, Switzerland. GFAP and NfL data was available for 12 HCs and 46 patients with MS.

MRI acquisition and analyses

MRI was used to gain volumetric data and as an anatomical reference for PET. MRI scans have been performed at Turku PET Centre or at Turku University Central Hospital with either 3T Ingenuity TF PET/MRI System scanner (n(MS)=20, n(HC)=6) or 3T Ingenia (n(MS)=27, n(HC)=4) (Philips

Table 2 MRI volumetric and [^{11}C]PK11195-DVR values of patients with MS and HCs

	HC	MS	P value*	PMS	RRMS	P value*
Volume (cm^3)						
n	10	47		17	30	
Brain	1150 (93.1)	1140 (114)	0.80	1100 (118)	1160 (108)	0.081
WM (HC), NAWM (MS)	467 (44.2)	448 (62.0)	0.36	413 (62.0)	467 (53.2)	**0.0028**
Thalamus	15.0 (1.17)	13.5 (1.98)	**0.025**	11.9 (1.41)	14.4 (1.68)	**<0.0001**
T1 lesion		2.89 (1.4–11.3)		15.8 (7.5–28.2)	1.76 (1.2–3.8)	**<0.0001**
T2 lesion		9.06 (2.7–19.3)		22.8 (16.7–31.8)	3.35 (2.2–9.5)	**<0.0001**
Number of PRLs†		1 (0–3)		3 (0.5–8.5)	1 (0–2)	**0.012**
DVR						
n	9	44		14	30	
Brain	1.19 (0.022)	1.19 (0.025)	0.64	1.21 (0.024)	1.18 (0.021)	**0.0006**
WM (HC), NAWM (MS)	1.18 (0.049)	1.20 (0.046)	0.30	1.24 (0.037)	1.18 (0.033)	**<0.0001**
Thalamus	1.32 (0.032)	1.34 (0.08)	0.55	1.39 (0.11)	1.32 (0.054)	**0.005**
T1 lesion		1.15 (1.1–1.2)		1.14 (1.1–1.3)	1.15 (1.1–1.2)	0.91
T2 lesion		1.13 (1.1–1.2)		1.12 (1.1–1.3)	1.14 (1.1–1.2)	0.73
T1 lesion rim (0–2 mm)		1.18 (0.08)		1.21 (0.09)	1.16 (0.07)	0.057
T1 perilesional area (2–6 mm)		1.18 (0.07)		1.23 (0.05)	1.15 (0.06)	**0.0003**

Continuous variables are presented as median and quartiles (Q1–Q3) or mean and standard deviation (SD) unless specified otherwise.
*From comparison of HC and MS or PMS and RRMS with two-tailed T-test or Mann-Whitney U test (T1 and T2 volumes, and DVRs and number of PRLs).
†4 values missing in PMS.
DVR, distribution volume ratio; HC, healthy control; MS, multiple sclerosis; NAWM, normal-appearing white matter; PMS, progressive multiple sclerosis; PRL, paramagnetic rim lesion; RRMS, relapsing-remitting multiple sclerosis.

Healthcare, Cleveland, Ohio, USA). Axial T2, 3D fluid-attenuated inversion recovery, 3DT1 with and without gadolinium, and optimised 3D gradient echo (GRE) sequences with spatial resolution of 1×1×1 mm were obtained. GRE sequence was available for all RRMS (n=30) and a proportion of patients with PMS (n=13). An 8-channel SENSE head coil was used in all MRI scans. A more detailed description of the applied MRI methods has been provided previously.[20] Quantitative susceptibility mapping (QSM) was used to detect paramagnetic rim lesions (PRLs). In brief, GRE images were postprocessed using MEDI Toolbox, visually inspected, and region of interest (ROI) masks were created for iron rims using ITK-SNAP V.4.2.0. PRLs were determined according to the North American Imaging in Multiple Sclerosis Cooperative Consensus Statement[21] for radiological definition of white matter PRLs.

The following ROIs were included in the study: brain, NAWM, thalamus, T1 and T2 lesions, T1 perilesional area and T1 rim. Lesion masks for the T1 and T2 lesion ROIs were created using a semi-automated method including visual inspection and manual edition of the masks.[22] ROI masks for NAWM (White matter-T2 lesions), T1 perilesional area (2–6 mm) and lesion rim (0–2 mm) were created as described earlier.[22] Freesurfer software was used to segment other included ROIs. After creating ROIs, the volumes were obtained as previously described.[23]

Translocator protein positron emission tomography (TSPO-PET) acquisition and processing

TSPO-PET using [^{11}C]PK11195-tracer was performed using an ECAT HRRT scanner (CTI/Siemens, resolution 2.5 mm) at Turku PET Centre as previously described.[22] The synthesis of the radioligand was performed at Turku PET Centre as previously described.[3 23] The mean (SD) injected dose was 476 (41.8) MBq in MS and 499 (8.7) MBq in HCs (p=0.019). The postprocessing was performed as previously described.[23] To quantify specific [^{11}C]PK11195 binding reflecting glial activation, distribution volume ratio (DVR) was calculated using the Logan method and a supervised cluster algorithm[24 25] (SuperPK software, SVCA classification) as previously described.[22]

Statistical analysis

Statistical analyses were performed using GraphPad Prism V.9.5.0 (GraphPad Software Inc., San Diego. CA). Continuous variables are presented as median and quartiles (Q1–Q3) or mean and SD. Normality was assessed using visual inspection, Q-Q plot and Shapiro-Wilk test. Depending on the normality, either an unpaired t-test or Mann-Whitney U test was used to assess group differences. Fisher's exact test was used for categorical variables. Linear regression modelling using R V.4.4.0 was used to assess the association between CHI3L1 and demographical, clinical, volumetric, [^{11}C]PK11195-DVR, log(NfL) and log(GFAP) values among all patients with MS. First, univariate linear regression was performed with all possible observations from each variable. Second, a multivariate stepwise linear regression model was built. The model building started with a model without any predictors, and in each step, the most suitable variable according to Bayesian information criterion (BIC) was added to the model. In this model, all variables used in the univariate model were considered, and only patients with available PET results were included. Log(CHI3L1–10) was used as a response in all models as non-transformed values led to non-normality of residuals. To assess the associations separately in RRMS and SPMS cohorts and in CHI3L1 low and high subgroups (divided based on the median CHI3L1 concentration of the whole MS group (20.8 ng/mL)), correlation analyses were performed, as the assumptions of the linear regression model were not fulfilled in all subgroups. Correlations were evaluated using Spearman correlation since CHI3L1 concentration was not normally distributed. Multiple linear regression was performed to evaluate the effect of covariates age and brain DVR on CHI3L1 concentration, which was

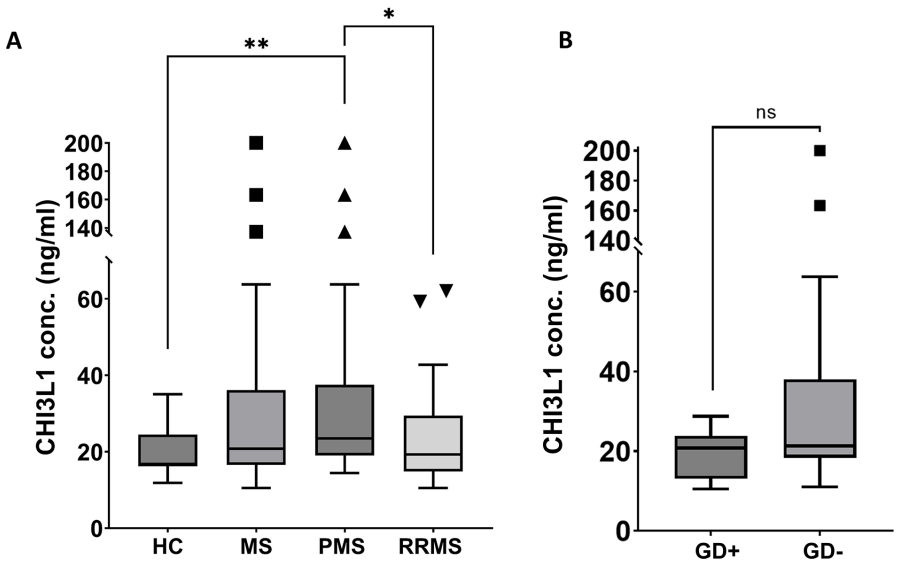

Figure 1 Plasma CHI3L1 concentration of patients with MS and HCs. (A) CHI3L1 levels were higher in PMS versus HC and PMS versus RRMS (Mann-Whitney U test). (B) There was no difference in the CHI3L1 concentration between patients with and without gadolinium-enhancing lesions. The CHI3L1 concentration was measured using ELISA. The inner line in the box represents the median of the data. The ends of the box indicate the upper (Q3) and lower (Q1) quartiles. The whiskers mark the 75th percentile +1.5* IQR values and the 25th percentile – 1.5 * IQR. Values greater/lower than the whiskers are marked as outliers and are shown as individual points. n(HC)=17, n(MS)=55, n(PMS)=25, n(RRMS)=30, n(Gadolinium+)=7, n(Gadolinium-)=36. CHI3L1, chitinase-3-like protein 1; HC, healthy control; MS, multiple sclerosis; PMS, progressive MS; RRMS, relapsing-remitting MS. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

converted to a form log(CHI3L1-10) to fulfil the normality assumptions. For all analyses, p<0.05 was considered significant and all tests were two-tailed.

RESULTS

Characteristics of the study cohort

Most of the patients with MS and HCs were females (67% and 65%, respectively). The age or BMI did not significantly differ between MS and HC. Patients with PMS were older, had a longer disease duration, higher EDSS and multiple sclerosis severity score (MSSS), and a lower BMI compared with RRMS. A total of seven patients with MS (n(PMS)=2, n(RRMS)=5) had gadolinium-enhancing lesions at study onset. 22% of study patients had a relapse during the year preceding sampling (n(PMS)=1, n(RRMS)=11). Characteristics of the study cohort are shown in table 1.

There was no significant difference in the whole brain volume between MS and HCs, but the thalamus volume was smaller in MS compared with HC (p=0.025; table 2). Patients with PMS had smaller NAWM (p=0.0028) and thalamus (p<0.0001) volumes, larger T1 (p<0.0001) and T2 (p<0.0001) lesion volumes, and greater number of PRLs (p=0.012) compared with RRMS. Overall, 10 patients with PMS and 16 with RRMS had one or more PRL.

The brain (p=0.033) and NAWM (p=0.0026) DVRs were higher in PMS compared with HCs but not in all MS compared with HCs (table 2, online supplemental figure 1). Furthermore, when PMS and RRMS cohorts were compared, the brain (p=0.0006), NAWM (p<0.0001) and thalamus (p=0.005) DVRs were higher in PMS. There were no significant differences in T2 and T1 lesion DVRs or within 0–2 mm lesion rim DVR between PMS and RRMS. However, DVR within the perilesional area was higher in PMS compared with RRMS (p=0.0003).

Blood chitinase-3-like protein (CHI3L1), glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL) concentrations in patients with multiple sclerosis (MS) and healthy controls (HCs)

The median plasma CHI3L1 concentration was increased in PMS compared with HCs (p=0.0055) and RRMS (p=0.0488) (table 1 and figure 1). The CHI3L1 concentration was slightly, although not statistically significantly, increased also among all MS compared with HCs (p=0.0675). There were no differences in CHI3L1 levels between females and males among MS or HCs (p=0.88 and p=0.51, respectively, data not shown). Additionally, there were no significant differences in CHI3L1 levels between treated and untreated patients with MS (p=0.54, data not shown) or between patients with or without gadolinium positive lesions (p=0.31, figure 1). Furthermore, no differences in CHI3L1 levels were observed when the RRMS cohort was divided into two groups, that is, RRMS active and RRMS inactive, based on disease activity within the previous year (p=0.63, table 3). Here, the activity assessment was based on the occurrence of relapses during the year preceding blood sampling and the presence of gadolinium-enhancing lesions at study MRI.

Serum GFAP (p=0.0004) and NfL levels (p=0.0098) were higher in MS compared with HCs (table 1). Moreover, both GFAP (p=0.0049) and NfL (p=0.0375) concentrations were higher in PMS compared with RRMS. In addition, NfL concentration was higher in active patients compared with inactive patients (p=0.021, table 3).

Association between chitinase-3-like protein (CHI3L1) and demographic and clinical parameters

Among all patients with MS, higher CHI3L1 levels associated with older age (standardised estimate 0.93, 95% CI 0.37 to 1.48, p=0.002), longer disease duration (0.62, 0.03 to 1.21, p=0.039) and increased EDSS (0.70, 0.12 to 1.28, p=0.019) in

Table 3 Demographic, clinical and biomarker parameters in active and inactive RRMS subcohorts

	RRMS active	RRMS inactive	P value*
n	12	18	
Female, n (%)	8 (67)	13 (72)	>0.99
Age	42.2 (6.3)	42.2 (6.7)	0.99
BMI	30.1 (7.7)	30.1 (6.2)	0.99
Disease duration†	3.6 (0.25–12)	8.3 (2.8–13.7)	0.11
MSSS	4.0 (1.5–6.4)	1.2 (0.43–3.4)	**0.036**
ARR	0.44 (0.21–1.7)	0.27 (0.20–0.69)	0.21
EDSS	2.3 (0.8)	1.7 (1.1)	0.096
Gd+lesions n (%)	5 (42)	0 (0)	**0.033**
Relapse-sampling‡	0.49 (0.24–0.76)	7.1 (2.0–9.4)	**<0.0001**
DMT n (%)	5 (42)	9 (50)	0.72
CHI3L1 (ng/ml)	23.8 (12.8–30.9)	19.7 (15.1–28.1)	0.63
NfL (pg/ml)	10.8 (7.5–25.4)	6.9 (5.9–12)	**0.021**
GFAP (pg/ml)	101 (40)	89 (30)	0.35

Continuous variables are presented as median and quartiles (Q1–Q3) or mean and SD unless specified otherwise. RRMS active included patients with relapses during the year proceeding blood sampling or with gadolinium enhancing lesions at study MRI.
*Group comparisons were conducted using Fisher's exact test (categorical variables) and Mann-Whitney U test or two-tailed T-test (age, BMI, EDSS and GFAP). Significant p values are bolded.
†From diagnosis to sampling.
‡Time between last relapse and sampling (years).
ARR, annualised relapse rate; BMI, body mass index; CHI3L1, chitinase-3-like protein 1; DMT, disease modifying treatment; EDSS, expanded disability status scale; Gd, gadolinium; GFAP, glial fibrillary acidic protein; MS, multiple sclerosis; MSSS, multiple sclerosis severity score; NfL, neurofilament light chain; RRMS, relapsing-remitting multiple sclerosis.

univariate linear regression models (figure 2). In addition, BMI was one of the three predictors included in the final multivariate model explaining 33% of the variation of plasma CHI3L1 in all patients with MS. In RRMS, CHI3L1 correlated with BMI (Spearman correlation coefficient ρ=0.56, 95% CI 0.24 to 0.77, p=0.0012) but not with other parameters. In PMS, CHI3L1 did not correlate with BMI (p=0.31) or age (p=0.13), or any of the other studied demographical and clinical variables (online supplemental table 1). In HCs, there was a correlation between CHI3L1 and age (ρ=0.55, 0.081 to 0.82, p=0.023).

Association between chitinase-3-like protein (CHI3L1) and brain glial activation

Among all patients with MS and in PMS, higher CHI3L1 was associated with increased whole brain DVR (0.89, 0.23 to 1.55, p=0.010 and ρ=0.58 0.058 to 0.86, p=0.032, respectively), but not with DVRs from any of the other explored ROIs (figure 2 and online supplemental table 2). There were no significant correlations between CHI3L1 and DVRs in RRMS or in HCs (online supplemental table 2).

Since age correlated significantly with CHI3L1, we performed a multiple linear regression analysis using log(CHI3L1-10) as the dependent variable and age and brain DVR as explanatory variables to explore whether the correlation between CHI3L1 and brain DVR was driven by age. In PMS, the overall regression model explained 47% of the variation in plasma CHI3L1 (p=0.031, table 4). Based on the model, only brain DVR was a significant predictor of CHI3L1 concentration (p=0.026 for brain DVR, p=0.72 for age). Among all patients with MS, the model explained 21% of the variation in CHI3L1 (p=0.0081), although neither brain DVR nor age predicted CHI3L1 independently (p=0.079 and p=0.085, respectively).

Association between chitinase-3-like protein (CHI3L1) concentration and MRI measures

Among all patients with MS, higher CHI3L1 was associated with smaller brain (−0.75, −1.38 to −0.11, p=0.023) and thalamus volumes (−0.88, −1.50 to −0.25, p=0.007, figure 2). Similarly, in PMS, higher CHI3L1 correlated with smaller brain volume (ρ=−0.56, −0.83 to −0.095, p=0.021, online supplemental table 1). Additionally, higher CHI3L1 correlated with both brain and NAWM volumes (ρ=−0.48, −0.76 to −0.041, p=0.029 and ρ=−0.46, −0.75 to −0.025, p=0.035, respectively) among 21 patients with CHI3L1 concentration above the median CHI3L1 concentration of the whole MS group. Demographic data of the CHI3L1 high and low subgroups are presented in online supplemental table 3. There were no significant correlations between CHI3L1 concentration and volumetric variables among patients with RRMS or in HCs. In PMS, there was a trend towards a correlation between a higher number of PRLs and increased CHI3L1 (ρ=0.51, p=0.076, online supplemental table 1).

Association between chitinase-3-like protein (CHI3L1) and neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) levels

There were no associations between CHI3L1 and NfL and GFAP among all MS (figure 2) or among HCs, PMS and RRMS (online supplemental table 1). However, among patients in the CHI3L1 high subgroup, higher plasma CHI3L1 correlated with higher serum levels of both GFAP (ρ=0.62, 0.24 to 0.83, p=0.0028) and NfL (ρ=0.62, 0.24 to 0.83, p=0.0027).

DISCUSSION

The primary aim of the present study was to evaluate the association between blood CHI3L1 and glial activation measured with TSPO-PET. We demonstrate here an association between increased plasma CHI3L1 and TSPO-PET measurable glial activation in patients with MS. The correlation between smaller brain volume and higher CHI3L1 among all and progressive patients with MS provides further evidence that CHI3L1 might reflect smouldering inflammation-related MS pathology.

In the clinical setting, MS most frequently manifests as RRMS at the time of diagnosis, while a small proportion of patients have a primary progressive clinical phenotype (PPMS). The majority of patients with initial RRMS disease acquire secondary progression typically after the age of 45 years. Despite these clinically well-recognised disease courses, there is significant heterogeneity in the severity of the disease and the underlying pathological mechanisms. It is now well established that in most patients, both adaptive immune mechanisms driving immune cell trafficking from the periphery into the CNS, and CNS-contained innate immune activation mechanisms drive the disease from the onset. While the focal inflammatory activity can be sensitively captured using conventional MRI, the CNS-contained inflammation is significantly harder to assess in patients in vivo. Advanced imaging methodology, such as susceptibility-weighted MRI for detection of PRLs and TSPO-PET for glial activation, has shown promise in capturing signs of diffuse CNS-contained pathology.[26] In addition, soluble biomarkers for microglial and astrocytic activation, including GFAP and CHI3L1, were considered markers for smouldering MS in a recent consensus statement.[27]

Multiple studies have found higher CHI3L1 blood levels in PMS compared with both HC and RRMS.[9 17 18] Aligning with these

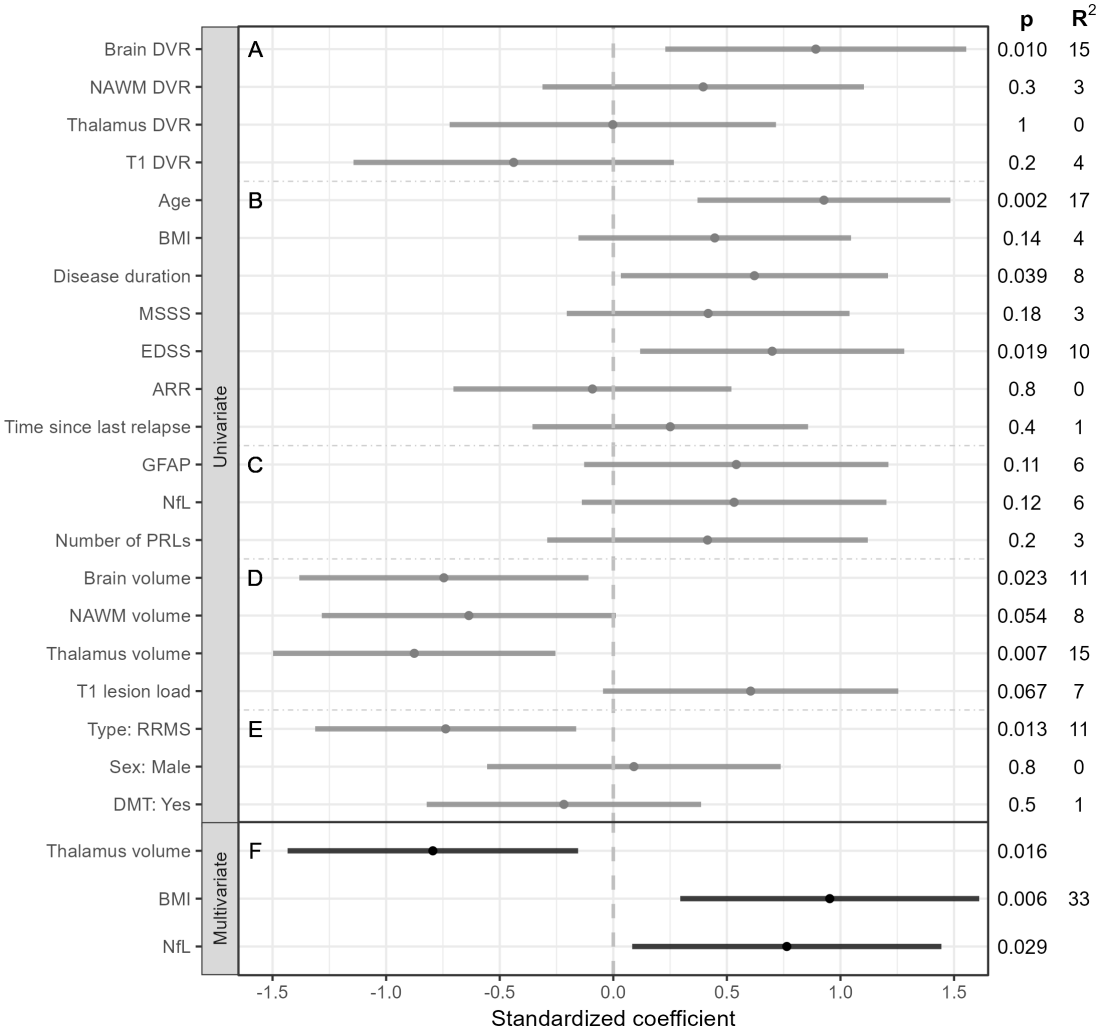

Figure 2 Forest plot illustrating the linear regression modelling of CHI3L1 with translocator protein positron emission tomography (TSPO-PET)-related, demographical and clinical, biomarker and volumetric variables among all MS. (A–E) Univariate regression model included all possible observations for each variable A. n=44, B&E. n=55, C. n(GFAP and NfL)=46, and n(iron rims)=43, D. n=47. In the univariate model, brain DVR (A) was a significant predictor of CHI3L1 concentration. When demographical and clinical variables (B&E) were considered, age, disease duration, EDSS and disease type predicted CHI3L1 concentration significantly. However, of the studied biomarkers (C), none predicted CHI3L1 concentration significantly. The brain and thalamus volume (D) had a significant inverse effect on CHI3L1. (**F**) Multivariate stepwise linear regression model included only those patients with available TSPO-PET imaging (n=44). The model building started with a model without any predictors, and in each step, the most suitable variable according to Bayesian information criterion (BIC) was added to the model. All variables that were used in univariate models were considered when the multivariate model was built. The final multivariate regression model included thalamus volume, BMI and NfL, and it explained 33% of the variance in CHI3L1 among all MS. The dots represent standardised regression coefficients, and the lines represent the confidence intervals of these estimates. Log(CHI3L1-10) was used as a response variable in all models to gain normality of residuals. Logarithm transformation was used for T1 lesion load, NfL and GFAP. ARR, annualised relapse rate; BMI, body mass index; CHI3L1, chitinase-3-like protein 1; DMT, disease modifying treatment; DVR, distribution volume ratio; EDSS, expanded disability status scale; GFAP, glial fibrillary acidic protein; MSSS, multiple sclerosis severity score; NfL, neurofilament light chain; PRL, paramagnetic rim lesion.

studies, our study demonstrated higher plasma CHI3L1 in PMS compared with HC and RRMS and an association between longer disease duration and increased CHI3L1. Furthermore, we found an association between higher CHI3L1 and increased EDSS among all patients with MS. This is in line with a previous study showing a similar correlation between EDSS and plasma CHI3L1 in a mixed

Table 4 Results of the multiple linear regression analysis exploring the effect of brain DVR and age on plasma CHI3L1 in MS and in PMS

	PMS				MS			
Variable	Estimate	SE	95% CI (asymptotic)	P value*	Estimate	SE	95% CI (asymptotic)	P value*
Brain DVR	31.8	12.3	4.70–58.9	**0.026**	12.6	7.0	–1.5–26.7	0.079
Age	0.012	0.033	–0.06–0.084	0.72	0.036	0.02	–0.0052–0.078	0.085

*Multiple linear regression with log(CHI3L1-10) as dependent variable and brain DVR and age as independent variables. The overall regression model was statistically significant F(2, 11)=4.83, p=0.031 in PMS and F(2, 41)=5.43, p=0.0081 in MS. Total number of subjects was n(PMS)=14 and n(MS)=44.
DVR, distribution volume ratio; MS, multiple sclerosis; PMS, progressive MS.

cohort consisting of both relapsing and progressive patients,[18] whereas others have observed either no correlation[9] or a correlation in patients with PMS.[28] In our study, CHI3L1 did not correlate with EDSS in PMS, which could be explained by our rather small PMS cohort.

At the moment, there are only a few studies that have studied the association of blood CHI3L1 with volumetric MRI parameters. We found an association between increased plasma CHI3L1 and smaller brain volume among all patients with MS and in PMS. In all MS, CHI3L1 was also associated with thalamus volume. On the other hand, CHI3L1 did not associate with lesion load. Partly in line with this, a previous study found no correlation between plasma CHI3L1 and brain parenchymal fraction or lesion loads in PPMS.[18] The results regarding brain volume and CSF CHI3L1 are also contradictory.[10 12 29 30] In the future, longitudinal data might clarify the association between CHI3L1 and brain volume loss.

In recent years, CHI3L1 has drawn attention as a soluble marker for glial activation.[9 11 12 26] The present study provides novel in vivo evidence about this. In PMS, increased plasma CHI3L1 correlated with higher brain DVR indicating increased glial activation and density. Additionally, brain DVR was associated with CHI3L1 among all patients with MS. Interestingly, we observed a non-significant association between the number of PRLs and CHI3L1 in PMS. Similar association has been previously found with iron rims and CSF CHI3L1.[30] These results suggest that CHI3L1 reflects pathological processes driving MS progression, but because of our small PMS cohort, the results must be further validated.

Based on our results and previous studies, focal inflammatory activity does not seem to affect blood CHI3L1 levels. We found no differences in plasma CHI3L1 between RRMS active and inactive groups. In addition, CHI3L1 did not correlate with relapse-associated variables. Similarly, in previous studies, serum CHI3L1 did not associate with T1 gadolinium enhancing and new or enlarged T2 lesions[31] or alter between relapse and remission states.[18] This strengthens the potential of CHI3L1 as a marker for smouldering inflammation. Accordingly, a recent study showed that in the PPMS cohort with no signs of focal inflammation, serum CHI3L1, but not GFAP or NfL, was associated with future EDSS change.[32]

The interpretation of brain-derived biomarker levels in blood can be complicated by several factors, such as peripheral production, age and body weight. Increased CHI3L1 levels have been found not only in nervous system diseases but also in various types of cancers,[8] and non-cancerous diseases such as asthma and COPD,[33] atherosclerosis,[34] diabetes[35] and osteoarthritis.[36] Therefore, the occurrence of systemic comorbidities could affect the concentration in peripheral blood. The correlation of CHI3L1 with age both among patients with MS and HCs might have affected the difference in plasma concentration of CHI3L1 in PMS compared with HCs and RRMS due to the older age of patients with PMS. However, there was no significant correlation between CHI3L1 and age in PMS, and the association between brain DVR and CHI3L1 observed in PMS remained significant when age was taken into account. Contrary to the previously observed inverse correlation of BMI with GFAP and NfL,[37] we found a positive correlation between BMI and CHI3L1 in RRMS, but not in PMS. This might be related to the link between CHI3L1 and low-grade inflammation in obesity.[38] Together with the lack of correlation with brain imaging variables in RRMS, this suggests that CHI3L1 at levels comparable to HCs might be more related to peripheral factors. On the other hand, the current study suggests that in MS, the increase in blood concentration of CHI3L1 might be induced by brain pathology, since in PMS, CHI3L1 correlated with brain DVR and volume, and in the CHI3L1 high group, CHI3L1 associated with GFAP and NfL. In line with this, serum levels of CHI3L1 have been shown to correlate with GFAP and NfL in PPMS.[32] Additionally, we have previously shown that serum NfL associates with glial activation measured with TSPO-PET.[39] The combination of soluble and imaging biomarkers reflecting microglial and astrocytic activation and neuroaxonal damage might provide improved patient-level specificity on characteristics of CNS-compartmentalised inflammation. Related to this, utilisation of a 'Glia score' calculated based on serum CHI3L1, NfL and GFAP has been proposed.[28]

There are some limitations in this study. We used commercial ELISA to measure plasma CHI3L1 levels, which can be considered a weakness, although ELISA has been widely used in previous CHI3L1 studies.[9 11 17 18 28 40] However, as the concentrations of most brain-derived biomarkers in the blood are extremely low, ultrasensitive methods, such as SIMOA, are nowadays usually used in measuring, for example, NfL and GFAP. Non-commercial SIMOA assay for CHI3L1 has already been used by a recent study to gain a wider dynamic range and reduce the final variability of the experiment.[32] Due to the exploratory nature of our study, we did not correct the analyses for multiple comparisons, which might have affected the results. Therefore, further studies with a larger PMS cohort and preferably blood CHI3L1 measurement using SIMOA are needed to validate the presented results. Furthermore, in addition to activated microglial cells, a subgroup of astrocytes is known to express the TSPO molecule, and thus TSPO-PET imaging cannot be considered to be fully specific for any given glial cell type. Novel radioligand development may help solve this issue in the future.

In conclusion, the present study shows increased levels of CHI3L1 in PMS and suggests an association between plasma CHI3L1 and disease pathology measured as brain atrophy and glial activity in progressive patients. Overall, these results suggest that blood CHI3L1 is a promising biomarker for MS progression-related smouldering inflammation.

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