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Chitinase 3-like 1: prognostic biomarker in clinically isolated syndromes

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Chitinase 3-like 1 (CHI3L1) has been proposed as a biomarker associated with the conversion to clinically definite multiple sclerosis in patients with clinically isolated syndromes, based on the finding of increased cerebrospinal fluid CHI3L1 levels in clinically isolated syndrome patients who later converted to multiple sclerosis compared to those who remained as clinically isolated syndrome. Here, we aimed to validate CHI3L1 as a prognostic biomarker in a large cohort of patients with clinically isolated syndrome. This is a longitudinal cohort study of clinically isolated syndrome patients with clinical, magnetic resonance imaging, and cerebrospinal fluid data prospectively acquired. A total of 813 cerebrospinal fluid samples from patients with clinically isolated syndrome were recruited from 15 European multiple sclerosis centres. Cerebrospinal fluid CHI3L1 levels were measured by enzyme-linked immunosorbent assay. Multivariable Cox regression models were used to investigate the association between cerebrospinal fluid CHI3L1 levels and time to conversion to multiple sclerosis and time to reach Expanded Disability Status Scale 3.0. CHI3L1 levels were higher in patients who converted to clinically definite multiple sclerosis compared to patients who continued as clinically isolated syndrome ($P = 8.1 \times 10^{-11}$). In the Cox regression analysis, CHI3L1 levels were a risk factor for conversion to multiple sclerosis (hazard ratio = 1.7; $P = 1.1 \times 10^{-5}$ using Poser criteria; hazard ratio = 1.6; $P = 3.7 \times 10^{-6}$ for McDonald criteria) independent of other covariates such as brain magnetic resonance imaging abnormalities and presence of cerebrospinal fluid oligoclonal bands, and were the only significant independent risk factor associated with the development of disability (hazard ratio = 3.8; $P = 2.5 \times 10^{-8}$). High CHI3L1 levels were associated with shorter time to multiple sclerosis ($P = 3.2 \times 10^{-9}$ using Poser criteria; $P = 5.6 \times 10^{-11}$ for McDonald criteria) and more rapid development of disability ($P = 1.8 \times 10^{-10}$). These findings validate cerebrospinal fluid CHI3L1 as a biomarker associated with the conversion to multiple sclerosis and development of disability and reinforce the prognostic role of CHI3L1 in patients with clinically isolated syndrome. We propose that determining cerebrospinal fluid chitinase 3-like 1 levels at the time of a clinically isolated syndrome event will help identify those patients with worse disease prognosis.

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Abbreviations: CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; IgG = immunoglobulin G; INDC = inflammatory neurological disease controls

Introduction

In a previous screening mass spectrometry-based proteomic study in pooled CSF samples from patients with clinically isolated syndromes (CIS), increased levels of chitinase 3-like 1 (CHI3L1) were found in CIS patients who converted to clinically definite multiple sclerosis compared with patients who continued as CIS (Comabella *et al.*, 2010). Measurement of CHI3L1 levels by enzyme-linked immunosorbent assay (ELISA) in individual CSF samples from patients with CIS resulted in similar findings, and increased levels of CHI3L1 at the time of the CIS event were associated with shorter time to clinically definite multiple sclerosis and disability progression during follow-up (Comabella *et al.*, 2010). In this initial study, the classification of CIS patients was performed according to extremes of outcome, and multiple sclerosis non-converters were patients with negative CSF oligoclonal bands and normal brain MRI at baseline and after 5 years of follow-up. On the other hand, multiple sclerosis converters were patients who also had positive oligoclonal bands and three or four Barkhof criteria in the baseline brain MRI (Comabella *et al.*, 2010). It remained unknown, however, whether CSF CHI3L1 findings in these two subgroups of CIS patients could also be extrapolated to any patient attending a multiple sclerosis outpatient clinic with a CIS suggestive of CNS demyelination. With this in mind, our main objective was to perform a validation study of CHI3L1 as a prognostic biomarker associated with the conversion to multiple sclerosis and disease severity in CSF samples from a large cohort of CIS patients from different multiple sclerosis centres. In a second part of the study, we investigated the cell source of CHI3L1 in brain tissue and CSF samples from patients with multiple sclerosis.

Patients and methods

Patients with clinically isolated syndromes

This is a multicentre collaborative study of patients presenting for the first time with monophasic neurological symptoms of the type observed in multiple sclerosis. Inclusion criteria were:

a CIS suggestive of CNS demyelination involving the optic nerve, brainstem, spinal cord, or other topography that was not attributable to other diseases; and entry window of 3 months since onset of symptoms. A total of 813 patients with CIS were recruited from 15 European multiple sclerosis centres. The study was approved by the corresponding local ethics committees and participants gave written informed consent.

MRI, CSF and clinical assessments were performed in each participating centre as part of their diagnostic workup. Patients were seen every 3 to 6 months and instructed to report any new or worsening of pre-existing symptoms. Brain MRI was performed during the first 5 months after the CIS event and repeated after 12 months and 5 years of follow-up. The number of Barkhof criteria (Barkhof *et al.*, 1997; Tintore *et al.*, 2008), number of T_2 lesions (recoded into three different categories: 0 lesions / 1–8 lesions / 9 or more lesions), and number of gadolinium enhancing lesions (recoded into two categories: 0 lesions / 1 or more lesions) in the baseline MRI were scored. Immunoglobulin G (IgG) oligoclonal bands were determined by isoelectric focusing combined with immunoblotting of matched serum and CSF sample pairs. Disability was evaluated according to the Expanded Disability Status Scale (EDSS) in each visit and only EDSS taken during stability periods was considered. EDSS scores collected during relapses were not included. The cut-off for defining the presence of disability was established when EDSS was superior or equal to 3.0 and maintained at two time points during the follow-up. An EDSS of 3.0 represents a clear milestone and corresponds to a patient who is fully ambulatory but has moderate disability in at least one system function or mild disability in three or four functional systems. Time of follow-up was calculated as the difference between the date of the last visit and the date of the CIS event. A diagnosis of conversion to multiple sclerosis according to Poser criteria was made when new symptoms occurred after an interval of at least 1 month and only when other diagnoses had been excluded (Poser *et al.*, 1983). A diagnosis of conversion to multiple sclerosis as defined by the 2005 McDonald criteria was made when patients fulfilled the MRI definitions for dissemination in time and space; or when patients had a second clinical attack (Polman *et al.*, 2005). Evidence of dissemination in space was provided by the presence of three of four MRI Barkhof parameters; or by the presence of at least two T_2 lesions together with oligoclonal bands. Dissemination in time was satisfied when at least one new T_2 lesion had appeared in the follow-up MRI (Polman *et al.*, 2005). Brain parenchymal fraction was calculated as the ratio of brain parenchymal tissue volume to the total intracranial volume. Data on the brain parenchymal fraction were available at baseline and after 1 and 5 years of follow-up in a subgroup of 149 CIS patients (all from the Centre d'Esclerosi Múltiple de Catalunya – Cemcat, Barcelona). The number of patients who received immunomodulatory treatment between the CIS event and the date of conversion to multiple sclerosis for converters, and during the follow-up time for multiple sclerosis non-converters was recorded.

Longitudinal clinical, CSF, and MRI information from CIS patients was prospectively acquired in the different participating centres. The 2005 McDonald criteria were retrospectively applied to this prospectively acquired data set.

A summary of demographic and clinical characteristics of the CIS cohort broken down by participating centre is presented in Table 1.

Control subjects

For comparison purposes, a cohort of 559 control subjects [438 non-inflammatory neurological disease controls (non-INDC) and 121 inflammatory neurological disease controls (INDC)] was also included in the study (Teunissen *et al.*, 2013). Demographic characteristics of the control cohort are shown in Table 1, and a description of the clinical diagnosis is detailed in Supplementary Table 1.

CSF sampling

CSF samples were collected by lumbar puncture for routine CSF diagnostics, which included number of cells, protein concentration, and determination of the IgG index and oligoclonal bands. The albumin CSF/serum ratio was studied in a subgroup of 59 CIS patients (40 patients from the University of Basel, Switzerland; and 19 patients from the University of Ulm, Germany).

CSF samples were centrifuged to remove cells, and the remaining volume was aliquoted and conserved at −80°C until used (Teunissen *et al.*, 2009). CSF characteristics of CIS patients are summarized in Table 1.

Quantification of CSF CHI3L1 levels

Levels of CHI3L1 were measured by ELISA using the METRA® EIA kit (Quidel) in undiluted CSF according to the manufacturers' recommendations. ELISA assays were performed in one single centre (Cemcat). Samples were run in duplicate in a blind manner, and the intra-assay and inter-assay variabilities were 2.6% and 3.8%, respectively.

CHI3L1 expression in human brain tissue

Paraffin-embedded brain samples of chronic active lesions from 15 patients with relapsing-remitting multiple sclerosis and 10 brain tissue samples from non-neurological controls were provided by the UK Multiple Sclerosis Tissue Bank and stained with haematoxylin and eosin and Klüver-Barrera for inflammation and demyelination assessment. Multiple sclerosis lesions were subsequently classified as chronic active with high inflammatory activity ($n = 10$) when hypercellularity, abundant inflammatory infiltration around blood vessels and at the edge of the lesion, and active demyelination were found. On the other hand, multiple sclerosis lesions were classified as chronic active with low inflammatory activity ($n = 5$) when hypocellularity, scarce perivascular inflammatory infiltration and no active demyelination were observed.

Tissue sections were immunostained with rabbit anti-YKL-40 (1:100, Quidel); antigen retrieval with Tris-EDTA (TE) buffer (pH = 9) was needed. Astrocytes were marked with rabbit anti-GFAP (1:900, Z0334, Dako), macrophages and microglia with mouse anti-CD68 (1:400, M0876, Dako), and T lymphocytes with rabbit anti-CD3 (1:100, A0452, Dako). Double immunostainings were visualized using EnVision™G|2 Doublestain System, Rabbit/Mouse (DAB + /Permanent Red;

Table 1 Demographic and clinical characteristics of CIS patients and controls with other neurological disorders included in the study

Patients/controls	*n*	Age (years)	Female/ male (% female)	Follow-up time (years)	CIS type[a] ON/BS/ SP/OT	% Treated patients[b]	CSF OB *n/N* (% positive)[c]	CSF cells (cells/μl)	CSF proteins (g/l)	*IgG index*
HC – Barcelona										
NC	7	33.7 (7.3)	3/4 (42.8)	5.5 (1.3)	1/2/3/1	42.9	6/7 (85.7)	13.3 (19.7)	41.3 (12.9)	0.7 (0.2)
C	23	27.8 (5.4)	18/5 (78.3)	6.7 (3.1)	4/8/10/1	13.0	17/23 (73.9)	6.8 (7.8)	35.9 (9.1)	0.9 (0.7)
Non-INDC	26	67.5 (15.6)	15/11 (57.7)	–	–	–	–	–	–	–
INDC	0	–	–	–	–	–	–	–	–	–
HRC – Madrid										
NC	20	34.3 (12.5)	15/5 (75.0)	4.1 (1.6)	7/6/2/5	10.0	11/20 (55.0)	3.7 (3.8)	NA	0.7 (0.3)
C	21	32.8 (6.3)	18/3 (85.7)	5.3 (2.6)	6/3/10/2	4.8	20/21 (95.2)	6.0 (7.4)	NA	1.0 (0.6)
Non-INDC	24	48.1 (14.3)	12/12 (50.0)	–	–	–	–	–	–	–
INDC	13	42.4 (15.3)	8/5 (61.5)	–	–	–	–	–	–	–
UULM - Ulm										
NC	12	38.9 (11.0)	8/4 (66.6)	2.2 (1.8)	8/1/2/1	0	10/12 (83.3)	4.7 (5.5)	44.9 (21.3)	0.7 (0.2)
C	17	29.9 (12.5)	11/6 (64.7)	2.5 (2.1)	7/5/3/2	0	17/17 (100)	9.7 (7.4)	41.5 (13.5)	0.9 (0.3)
Non-INDC	14	51.6 (13.5)	6/8 (42.8)	–	–	–	–	–	–	–
INDC	0	–	–	–	–	–	–	–	–	–
EUMC – Rotterdam										
NC	16	32.9 (7.0)	12/4 (75.0)	3.7 (2.3)	8/4/4/0	6.3%	8/16 (50.0)	7.5 (8.5)	32.1 (11.0)	1.0 (0.8)
C	13	32.5 (6.9)	11/2 (84.6)	5.7 (3.0)	7/3/1/2	61.5%	12/13 (92.3)	14.0 (16.1)	35.6 (12.7)	1.1 (0.9)
Non-INDC	29	49.5 (17.1)	15/14 (51.7)	–	–	–	–	–	–	–
INDC	1	28.0	1/0 (100)	–	–	–	–	–	–	–
GM – Madrid										
NC	13	34.5 (7.3)	12/1 (92.3)	3.2 (2.2)	6/2/4/1	0%	NA	5.2 (7.4)	26.8 (8.3)	0.8 (0.3)
C	9	34.1 (6.6)	7/2 (77.8)	3.2 (2.1)	2/2/2/3	55.6%	NA	6.0 (4.4)	26.9 (4.0)	0.8 (0.3)
Non-INDC	13	31.8 (9.0)	9/4 (69.2)	–	–	–	–	–	–	–
INDC	0	–	–	–	–	–	–	–	–	–
PH – Madrid										
NC	18	33.4 (7.0)	12/6 (66.7)	4.5 (1.1)	3/7/8/-	27.8%	15/17 (88.2)	3.8 (3.1)	40.1 (8.7)	0.7 (0.3)
C	15	33.9 (9.1)	11/4 (73.3)	5.0 (1.5)	2/7/4/2	13.3%	12/13 (92.3)	6.7 (6.7)	36.7 (12.8)	1.0 (0.5)
Non-INDC	23	43.0 (17.0)	8/15 (34.8)	–	–	–	–	–	–	–
INDC	5	50.0 (13.8)	1/4 (20.0)	–	–	–	–	–	–	–
HP – Toulouse										
NC	19	35.6 (11.9)	15/4 (78.9)	1.4 (1.2)	5/3/9/2	100%[d]	17/19 (89.5)	6.1 (6.8)	38.4 (16.8)	0.9 (0.5)
C	19	23.9 (7.8)	15/4 (78.9)	3.6 (1.2)	7/6/5/1	100	18/19 (94.7)	6.6 (6.7)	41.2 (10.2)	0.8 (0.3)
Non-INDC	14	41.9 (11.5)	10/4 (71.4)	–	–	–	–	–	–	–
INDC	1	57.0	1/0 (100)	–	–	–	–	–	–	–
OP – Milan										
NC	2	36.5 (3.5)	1/1 (50.0)	5.3 (2.2)	1/-/-/1	0	2/2 (100)	4.3 (2.1)	27.0 (17.0)	0.6 (0.2)
C	9	31.4 (6.8)	3/6 (33.3)	4.2 (2.6)	2/1/1/3[e]	0	7/8 (87.5)	6.9 (6.5)	33.6 (8.2)	1.1 (0.4)
Non-INDC	2	52.0 (33.9)	2/0 (100)	–	–	–	–	–	–	–
INDC	5	33.0 (4.0)	2/3 (40.0)	–	–	–	–	–	–	–
USB – Basel										
NC	16	38.4 (13.1)	14/2 (87.5)	3.7 (3.2)	8/2/2/4	43.8	11/16 (68.7)	4.6 (6.1)	34.1 (8.3)	0.8 (0.3)
C	24	33.8 (8.7)	18/6 (69.2)	6.3 (3.9)	6/4/8/6	20.8	22/24 (91.7)	10.8 (9.3)	44.0 (13.7)	1.1 (0.6)
Non-INDC	25	38.6 (11.4)	14/11 (56.0)	–	–	–	–	–	–	–
INDC	25	51.2 (17.6)	11/14 (44.0)	–	–	–	–	–	–	–
MUL – Lublin										
NC	1	62.0	1/0 (100)	2.2	-/-/1/-	0	0/1 (0.0)	1.0	28.0	NA
C	22	33.8 (8.5)	10/12 (45.4)	3.8 (2.6)	7/2/4/9	0	14/22 (63.6)	7.8 (11.3)	53.8 (44.6)	1.3 (0.2)
Non-INDC	20	35.7 (12.0)	3/17 (15.0)	–	–	–	–	–	–	–
INDC	3	43.7 (22.0)	0/3 (0.0)	–	–	–	–	–	–	–
CU – Prague										
NC	44	32.2 (9.7)	33/11 (75.0)	3.3 (1.0)	23/3/9/9	45.5	39/44 (88.6)	28.8 (30.3)	30.8 (11.7)	0.8 (0.5)
C	21	31.7 (10.4)	16/5 (76.2)	3.4 (0.9)	7/4/3/7	85.7	21/21 (100)	38.1 (41.1)	32.2 (9.6)	1.1 (0.7)
Non-INDC	35	31.9 (9.1)	25/10 (71.4)	–	–	–	–	–	–	–
INDC	9	33.5 (7.8)	5/4 (55.5)	–	–	–	–	–	–	–

(continued)

Table 1 Continued

Patients/controls	*n*	Age (years)	Female/ male (% female)	Follow-up time (years)	CIS type[a] ON/BS/ SP/OT	% Treated patients[b]	CSF OB *n/N* (% positive)[c]	CSF cells (cells/μl)	CSF proteins (g/l)	*IgG index*
MU – Graz										
NC	6	35.0 (9.9)	4/2 (66.7)	1.9 (0.6)	3/-/2/1	66.7	6/6 (100)	9.5 (5.0)	39.0 (12.5)	0.8 (0.3)
C	4	33.7 (13.2)	2/2 (50.0)	2.6 (0.4)	1/2/1/-	25.0	4/4 (100)	16.3 (20.0)	38.5 (11.0)	1.8 (0.8)
Non-INDC	15	28.9 (8.0)	8/7 (53.3)	–	–	–	–	–	–	–
INDC	5	32.0 (16.1)	1/4 (20.0)	–	–	–	–	–	–	–
CEMCAT – Barcelona										
NC	166	32.3 (8.4)	127/39 (76.5)	5.7 (3.7)	75/43/26/22	12.0	76/164 (46.3)	5.9 (23.1)	37.9 (14.5)	0.7 (0.5)
C	166	28.8 (7.3)	114/52 (68.7)	9.1 (3.3)	59/38/54/15	75.3	127/165 (76.9)	7.3 (10.9)	37.9 (17.0)	1.0 (0.6)
Non-INDC	93	41.5 (17.0)	53/34 (60.9)	–	–	–	–	–	–	–
INDC	6	33.3 (11.0)	4/1 (80.0)	–	–	–	–	–	–	–
IMU – Innsbruck										
NC	28	34.6 (10.1)	19/9 (67.8)	2.5 (1.4)	8/6/9/5	17.9	28/28 (100)	14.0 (14.8)	40.4 (13.0)	1.2 (0.7)
C	19	29.6 (7.5)	14/5 (73.7)	3.5 (1.3)	4/7/6/2	10.5	19/19 (100)	12.5 (11.3)	42.2 (13.3)	1.1 (0.7)
Non-INDC	58	50.1 (15.0)	38/20 (65.5)	–	–	–	–	–	–	–
INDC	48	47.0 (20.7)	17/31 (35.4)	–	–	–	–	–	–	–
KUH – Solna										
NC	26	39.3 (10.9)	20/6 (76.9)	2.9 (3.7)	7/5/7/7	0	14/26 (53.8)	4.7 (6.0)	NA	0.6 (0.2)
C	37	33.0 (8.3)	29/8 (78.4)	5.5 (3.1)	11/5/11/10	0	33/37 (89.2)	8.4 (6.4)	NA	0.9 (0.4)
Non-INDC	47	41.1 (13.1)	31/16 (65.9)	–	–	–	–	–	–	–
INDC	0	–	–	–	–	–	–	–	–	–
Summary										
NC	394	33.9 (9.7)	296/98 (75.1)	4.2 (3.2)	163/84/88/59	20.6	243/377 (64.4)	9.2 (19.7)	36.5 (13.8)	0.8 (0.5)
C	419	30.6 (8.1)	297/122 (70.9)	6.4 (3.7)	132/97/123/65	45.1	344/407 (84.5)	9.9 (15.2)	39.2 (18.6)	1.0 (0.6)
Non-INDC	438	43.6 (16.6)	249/183 (57.6)	–	–	–	–	–	–	–
INDC	121	44.7 (18.3)	51/69 (42.5)	–	–	–	–	–	–	–

Data are expressed as mean (standard deviation), except for the follow-up time which is expressed as median (interquartile range).

[a]Clinical presentation and data indicate the number of patients for each CIS type: ON = optic neuritis; BS = brainstem; SP = spinal; OT = other topographies.

[b]Percentage of patients who received immunomodulatory treatment between the CIS event and the date of conversion to multiple sclerosis for converters, and during follow-up time for non-converters. The variability in the percentage of treated CIS patients probably reflects the use of different criteria to initiate immunomodulatory treatment across multiple sclerosis centres. Of 792 CIS patients, 71 (9.0%) received corticosteroid treatment within the month prior to lumbar puncture (information was missing in 21 patients). Two of 813 CIS patients (0.2%) were receiving immunomodulators at the time of lumbar puncture. These two variables (treatment with corticosteroids and immunomodulators) did not remain significant in the multivariable Cox regression models.

[c]*n* = number of patients with positive oligoclonal bands / *N* = total number of patients in whom oligoclonal bands (OB) were determined (percentage of positive patients). Ten per cent of the patients included in the validation study from the CEMCAT cohort also participated in the previous proteomic study (Comabella *et al.*, 2010).

[d]Information on treatment not available for six patients.

[e]Information on CIS type not available for two patients.

Centre abbreviations: HC – Barcelona = Hospital Clínic, Barcelona; HRC – Madrid = Hospital Ramón y Cajal, Madrid; UULM – Ulm = University of Ulm, Ulm; EUMC – Rotterdam = Erasmus University Medical Centre, Rotterdam; GM – Madrid = Hospital Gregorio Marañón, Madrid; PH – Madrid = Hospital Puerta de Hierro, Madrid; HP – Toulouse = Hôpital Purpan, Toulouse; OP – Milan = Ospedale Policlinico, Milan; USB – Basel = University Hospital, University of Basel, Basel; MUL – Lublin = Medical University of Lublin, Lublin; CU – Prague = Charles University, Prague; MU – Graz = Medical University of Graz, Graz; CEMCAT – Barcelona = Centre d'Esclerosi Múltiple de Catalunya, Barcelona; IMU – Innsbruck = Innsbruck Medical University, Innsbruck; KUH – Solna = Karolinska University Hospital, Solna.

Converters (C) and non-converters (NC) refer to CIS patients who converted and who did not convert to multiple sclerosis by Poser criteria, respectively; NA = data not available.

K5361, Dako) according to the manufacturers' recommendations. Slides were counterstained using REAL Haematoxylin (S2020, Dako) and mounted in Glycerol Mounting Medium (C0563, Dako).

CHI3L1 expression in CSF cells

CHI3L1 expression was determined by flow cytometry in fresh CSF samples from five consecutive untreated multiple sclerosis patients and five controls (optic neuropathy, head trauma, arachnoid cyst, and two controls with neuropathies). The study was approved by the ethics committee of the Hospital Ramón y Cajal, Madrid.

The following monoclonal antibodies were used for cell surface staining: control mouse isotypes IgG1-FITC, IgG1-APC, anti-CD45-PerCP-Cy5.5, anti-CD3-APC, and anti-CD14-FITC (Becton Dickinson). For intracellular staining, a polyclonal rabbit antibody anti-human CHI3L1 (Quidel) and a purified rabbit IgG (Abcam) were biotinylated with sulfo-NHS-biotin (Pierce) following the manufacturers' instructions.

CSF samples were centrifuged at 500*g* for 15 min and cellular pellets resuspended in 100 μl of PBS, divided in two identical aliquots, and labelled with optimal concentrations of anti-CD45-PerCP-Cy5.5 and isotype controls conjugated with fluorescein isothiocyanate (FITC) and allophycocyanin (APC) or with anti-CD45-PerCP-Cy5.5, anti CD14-FITC and anti-CD3-APC for 30 min at 4°C. For intracellular labelling, cells were washed in saline and resuspended in 200 μl of Cytofix/Cytoperm™ (Becton Dickinson). After 20 min at 4°C, cells were washed in Perm/Wash buffer, pellets resuspended and labelled with 1 μg of rabbit IgG-biotin or anti CHI3L1-biotin

for 1 h at 4°C and washed. Finally, cells were incubated with streptavidin-PE for 45 min at 4°C.

Samples were analysed on a standard FACSCanto II instrument (Becton Dickinson). An initial region was set around cells expressing intermediate to high CD45 with low to intermediate side scatter (P1) and then a second region was set on the forward/side scatter dot plot to exclude debris or apoptotic cells (P2). Only cells included in P1 and P2 regions were accepted for analysis. Monocytes were then selected by gating side scatter versus CD14 expression to identify CD14 low ($CD14_{low}$) and CD14 high ($CD14_{high}$) subsets, and T cells by gating side scatter versus CD3 expression. Representative examples of gating strategy and of anti-CHI3L1 and control isotype staining in the three cell subsets are shown in Supplementary Fig. 1. The specificity of anti-CHI3L1 antibody staining was assessed by means of blocking experiments incubating 1 µg of anti-CHI3L1 antibody with 2 µg of human recombinant CHI3L1 protein (TP303769 Origene) for 30 min at 4°C before cell labelling, and resulted in a clear inhibition of CHI3L1 staining (Supplementary Fig. 2).

Statistical analysis

Statistical analysis was performed by using the SPSS 17.0 package (SPSS) for MS-Windows. Considering that CSF CHI3L1 levels were not normally distributed and age-dependent (Spearman rank correlation coefficient with age: 0.330, *P*-value: 5.2×10^{-36}; Supplementary Fig. 3), a Mann-Whitney's U-test was used to compare age-adjusted CSF CHI3L1 levels between CIS patients and controls with other neurological disorders. Receiver operating characteristic (ROC) curve analyses were used to determine the best cut-off value based on CSF CHI3L1 levels. Univariate and multivariable Cox proportional hazard regression models including number of Barkhof criteria at baseline MRI, presence of IgG oligoclonal bands, treatment, and age at CIS onset as covariates were used to evaluate the association between CSF CHI3L1 levels and time to multiple sclerosis based on Poser or McDonald criteria, and time to reach EDSS 3.0. Time to multiple sclerosis based on Poser and McDonald criteria, and time to reach EDSS 3.0 in patients with high and low CSF CHI3L1 levels were assessed by Kaplan–Meier survival analysis with Log Rank test.

A Mann-Whitney's U-test was used to evaluate significant differences in age-adjusted CSF CHI3L1 levels between patients with different categories of T_2 lesion burden and brain inflammation, and differences in CHI3L1 expression by CSF CD3+ T cells and CD14+ monocytes between multiple sclerosis patients and controls. Partial correlations adjusted for age were used to assess the relationship between CSF CHI3L1 levels and (i) the albumin CSF/serum ratio; (ii) CSF characteristics such as number of cells, protein concentration, and IgG index; and (iii) change in brain parenchymal fraction at 1 and 5 years of follow-up.

Results

Clinical characteristics of the CIS cohort

Mean and median follow-up times of patients with CIS were 5.4 and 4.6 years, respectively. Of 813 patients, 419 (51.5%) converted to multiple sclerosis by Poser criteria and 394 remained as CIS during follow-up. A total of 486 patients (60%) converted to multiple sclerosis by McDonald criteria and 327 continued as CIS. The most frequent clinical presentation of patients with CIS was optic neuritis (36.4%), followed by spinal (26.0%), brainstem (22.3%), and CIS of other topography (15.3%). IgG oligoclonal bands were positive in 587 (75%) of 784 patients. Of 609 patients, 96 (16%) reached an EDSS of 3.0 during follow-up. A total of 269 patients (33.3%) received immunomodulatory treatment between the CIS event and the date of conversion to multiple sclerosis for converters, and during follow-up time for non-converters. Clinical characteristics of CIS patients stratified by participating centre and conversion to multiple sclerosis are detailed in Table 1.

CSF CHI3L1 levels are increased in patients with CIS who convert to clinically definite multiple sclerosis

As a first step, we compared CSF CHI3L1 levels between the whole cohort of CIS patients and control subjects with other neurological disorders. As depicted in Fig. 1A, CHI3L1 levels were higher in CIS patients than in non-inflammatory controls ($P = 1.8 \times 10^{-27}$). However, the highest CHI3L1 levels were observed in inflammatory controls, and differences were statistically significant when compared with patients with CIS ($P = 3.2 \times 10^{-9}$) and non-inflammatory control subjects ($P = 1.9 \times 10^{-19}$). Further stratification of the CIS group revealed significantly higher CSF CHI3L1 levels in patients with CIS who converted to clinically definite multiple sclerosis compared to patients who remained as CIS ($P = 8.1 \times 10^{-11}$; Fig. 1B).

CSF CHI3L1 levels are an independent risk factor for conversion to multiple sclerosis and development of disability

Supplementary Fig. 4 shows the areas under the ROC curve (AUC) of CSF CHI3L1, which were significant for time to multiple sclerosis based on Poser criteria [AUC = 0.59; 95% confidence interval (CI) 0.55–0.63; $P = 1.4 \times 10^{-5}$] and McDonald criteria (AUC = 0.61; 95% CI 0.57–0.65; $P = 9.7 \times 10^{-8}$), and for time to EDSS 3.0 (AUC = 0.70; 95% CI 0.64–0.76; $P = 5.1 \times 10^{-10}$). A CHI3L1 value of 170 ng/ml resulted in the best cut-off point to classify CSF protein levels into high and low. For this cut-off, sensitivity and specificity were 51.8% and 63.7%, respectively for time to multiple sclerosis by Poser criteria; 51.2% and 66.1% for time to multiple sclerosis by McDonald criteria; and 74.0% and 60.0% for time to EDSS 3.0.

A total of 360 CIS patients (44.3%) had CHI3L1 levels above the cut-off value. We first investigated the

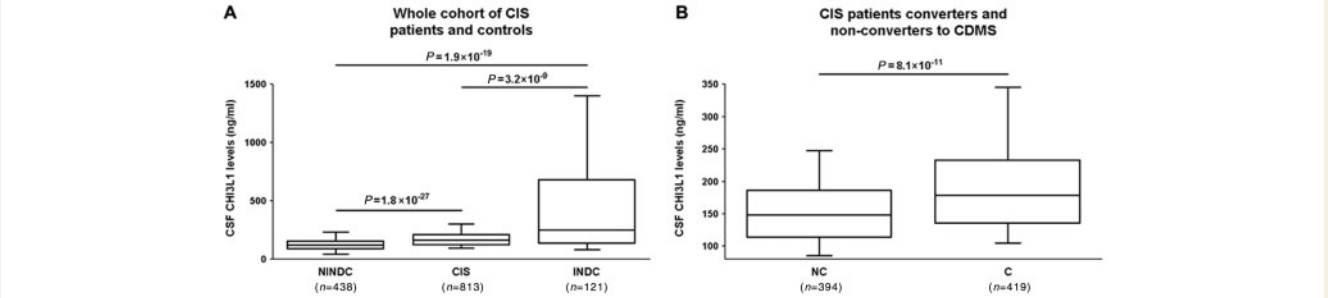


Figure 1 Comparison of CSF CHI3L1 levels among groups. Boxplots showing CSF levels of CHI3L1 in the whole CIS group and inflammatory and non-inflammatory neurological controls (**A**), and in CIS patients who converted to clinically definite multiple sclerosis (CDMS) and patients who continued as CIS (**B**). CSF CHI3L1 levels were age-adjusted and then compared among groups by a Mann-Whitney U-test. Parentheses indicate number of individuals included within each group. CIS = whole CIS cohort; C = CIS patients who converted to clinically definite multiple sclerosis (clinically definite multiple sclerosis); NINDC = non-inflammatory neurological disease controls; INDC = inflammatory neurological disease controls; NC = CIS patients who did not convert to clinically definite multiple sclerosis during follow-up.

association between CSF CHI3L1 levels stratified according to the cut-off value and time to conversion to multiple sclerosis and time to reach EDSS 3.0 in univariate and multivariable Cox regression models adjusted by the number of Barkhof criteria at baseline MRI, presence of oligoclonal bands, treatment, and age at CIS onset. As shown in Fig. 2, CSF CHI3L1 levels were an independent risk factor for conversion to multiple sclerosis based on Poser criteria [hazard ratio (HR) = 1.69; $P = 1.1 \times 10^{-5}$] or McDonald criteria (HR = 1.61; $P = 3.7 \times 10^{-6}$). Interestingly, CSF levels of CHI3L1 were the only significant independent risk factor associated with the development of disability (HR = 3.82; $P = 5.3 \times 10^{-8}$).

High CSF CHI3L1 levels are associated with shorter time to multiple sclerosis and more rapid development of disability

We next examined time to multiple sclerosis and time to reach EDSS 3.0 in patients with high and low CSF CHI3L1 levels by Kaplan–Meier survival analysis. As depicted in Fig. 3, high CSF CHI3L1 levels were associated with shorter time to multiple sclerosis based on Poser criteria (log-rank P-value = 3.2×10^{-9}) or McDonald criteria ($P = 5.6 \times 10^{-11}$). Similarly, when the time to reach an EDSS of 3.0 was evaluated, high CSF CHI3L1 levels were associated with more rapid development of disability ($P = 1.8 \times 10^{-10}$).

Overall, high CSF CHI3L1 levels were also associated with a worse prognosis in patients with CIS classified according to the presence or absence of IgG oligoclonal bands and MRI abnormalities (Supplementary Fig. 5). When CIS patients were classified according to treatment, high CSF CHI3L1 were associated with earlier disability progression in both untreated and treated patients; however, significant differences were lost in the treated group when time to multiple sclerosis was evaluated (Supplementary Fig. 6). Finally, time to reach an EDSS of 3.0 was shorter in CIS patients who converted to multiple sclerosis based on Poser or McDonald criteria and had high CSF CHI3L1 levels compared to non-converters (Supplementary Fig. 7).

CSF CHI3L1 levels are associated with brain MRI abnormalities at baseline and with the development of brain atrophy during follow-up

CSF CHI3L1 levels reflected the degree of brain inflammation and lesion burden in MRI scans performed at the time of the CIS event. As shown in Fig. 4A, CHI3L1 levels were significantly higher in patients with gadolinium enhancing lesions than in patients without enhancing lesions ($P = 1.4 \times 10^{-7}$), and in patients with nine or more T_2 lesions compared to patients with one to eight lesions ($P = 3.4 \times 10^{-4}$) and patients with no T_2 lesions ($P = 2.5 \times 10^{-5}$).

In the subgroup of CIS patients with data on brain atrophy during follow-up, CSF CHI3L1 levels correlated significantly with brain parenchymal fraction change after 1 year (r = 0.25; $P = 0.04$) and 5 years (r = 0.38; $P = 0.002$).

CHI3L1 levels in CSF are primarily brain derived and correlate with inflammatory CSF parameters

To evaluate the relationship between CSF CHI3L1 levels and blood-CSF barrier dysfunction, the albumin CSF/serum ratio was analysed in CSF samples from a subgroup of CIS patients. As illustrated in Fig. 4B, CHI3L1 protein levels were little influenced by the albumin CSF/serum ratio (r = 0.04; $P = 0.795$), indicating that CSF CHI3L1 protein is mainly brain-derived.

CSF CHI3L1 levels correlated weakly with CSF characteristics such as number of cells (r = 0.11; $P = 0.008$),

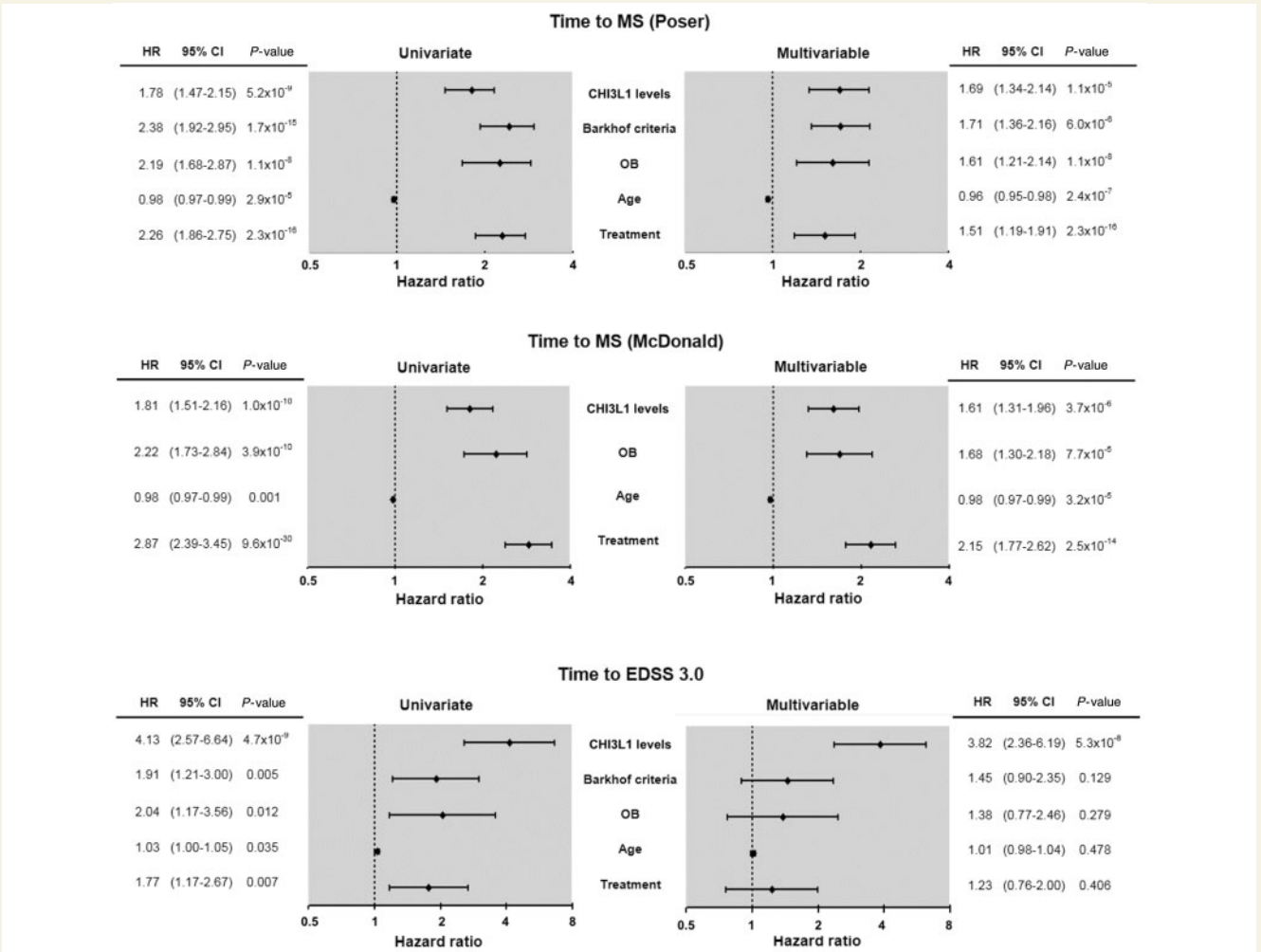
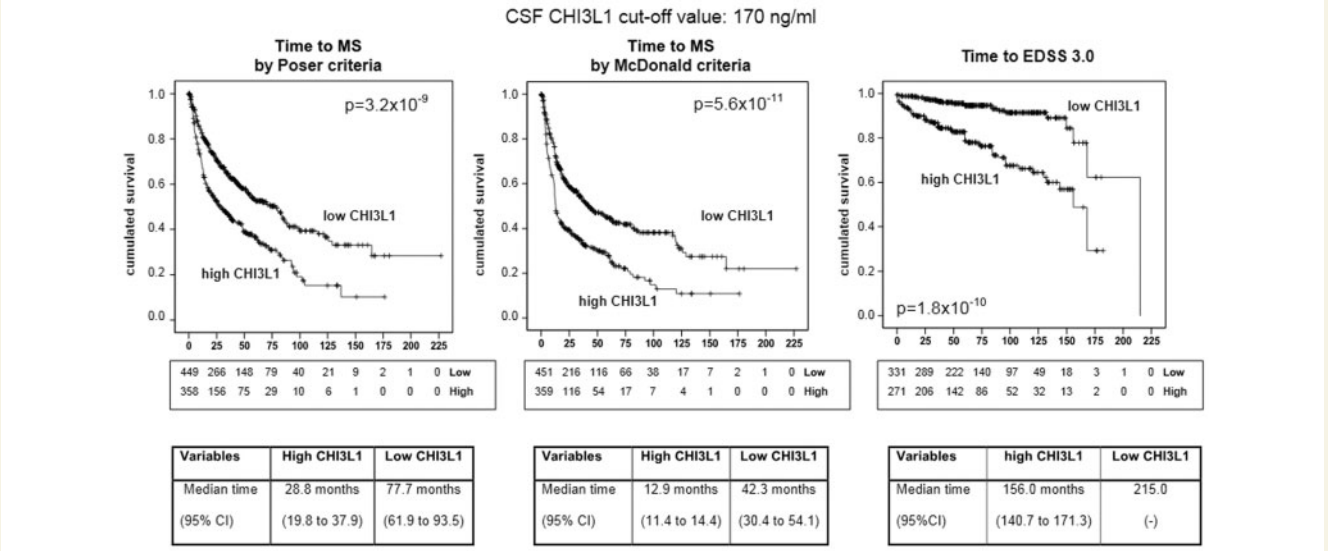


Figure 2 Analysis of the prognostic role of CSF CHI3L1 levels in CIS patients. Results of univariate and multivariable Cox regression analyses investigating the association between CSF CHI3L1 levels and time to conversion to multiple sclerosis (MS) and time to reach EDSS 3.0. For conversion to multiple sclerosis based on Poser criteria and time to EDSS 3.0, multivariable Cox regression model was adjusted by the number of Barkhof criteria at baseline MRI, presence of oligoclonal bands, treatment, and age at CIS onset. For conversion to multiple sclerosis based on McDonald criteria, considering that the number of Barkhof criteria is included in the McDonald criteria for conversion to multiple sclerosis, multivariable analysis was adjusted by the presence of oligoclonal bands, treatment, and age at CIS onset but not by baseline Barkhof criteria. CHI3L1 levels: CSF CHI3L1 levels stratified according to a cut-off value of 170 ng/ml. Barkhof criteria: number of Barkhof criteria recoded into two categories: 0, 1, 2 Barkhof criteria and 3, 4 Barkhof criteria. OB = presence or absence of IgG oligoclonal bands; Age = age at CIS onset. Treatment refers to whether patients received treatment between the CIS event and the date of conversion to multiple sclerosis for converters, and during follow-up time for non-converters.

protein concentration (r = 0.13; $P = 0.002$), and IgG index (r = 0.20; $P = 5.8 \times 10^{-6}$).

CHI3L1 is expressed by reactive astrocytes and macrophages/microglia from brain tissue and $CD14_{low}$ monocytes from CSF

As a last step, we investigated the CHI3L1 cell source in brain tissue and CSF from multiple sclerosis patients. In chronic active lesions with high inflammatory activity, CHI3L1 immunostaining was more intense and located at the edge of the lesions and in the demyelinated area (Fig. 5A and G), whereas in lesions with low inflammatory activity CHI3L1 immunostaining was less intense and mainly restricted to the demyelinated area (Fig. 5A and H). In contrast, non-neurological control brain samples were negative for CHI3L1 staining (Fig. 5A and I). Double immunostainings in lesions with high inflammatory activity revealed CHI3L1 expression by reactive astrocytes (GFAP+ cells; Fig. 5A and J) and macrophages/microglial cells (CD68+ cells; Fig. 5A and M). In lesions with low inflammatory activity, CHI3L1 expression was restricted to the cytoplasm of few macrophages/microglial cells (CD68+ cells; Fig. 5A and N). T lymphocytes (CD3+ cells) were



Variables	High CHI3L1	Low CHI3L1
Median time	28.8 months	77.7 months
(95% CI)	(19.8 to 37.9)	(61.9 to 93.5)

Variables	High CHI3L1	Low CHI3L1
Median time	12.9 months	42.3 months
(95% CI)	(11.4 to 14.4)	(30.4 to 54.1)

Variables	high CHI3L1	Low CHI3L1
Median time	156.0 months	215.0
(95%CI)	(140.7 to 171.3)	(-)

Figure 3 Kaplan-Meier curves for time to multiple sclerosis and time to EDSS 3.0 according to baseline CSF CHI3L1 levels classified into high and low based on a cut-off value of 170 ng/ml. Graphs show log-rank *P*-values. Numbers represent patients at risk for the different follow-up times. Tables indicate median times (95% CI) to multiple sclerosis and EDSS 3.0 in CIS patients with high and low CHI3L1 levels.

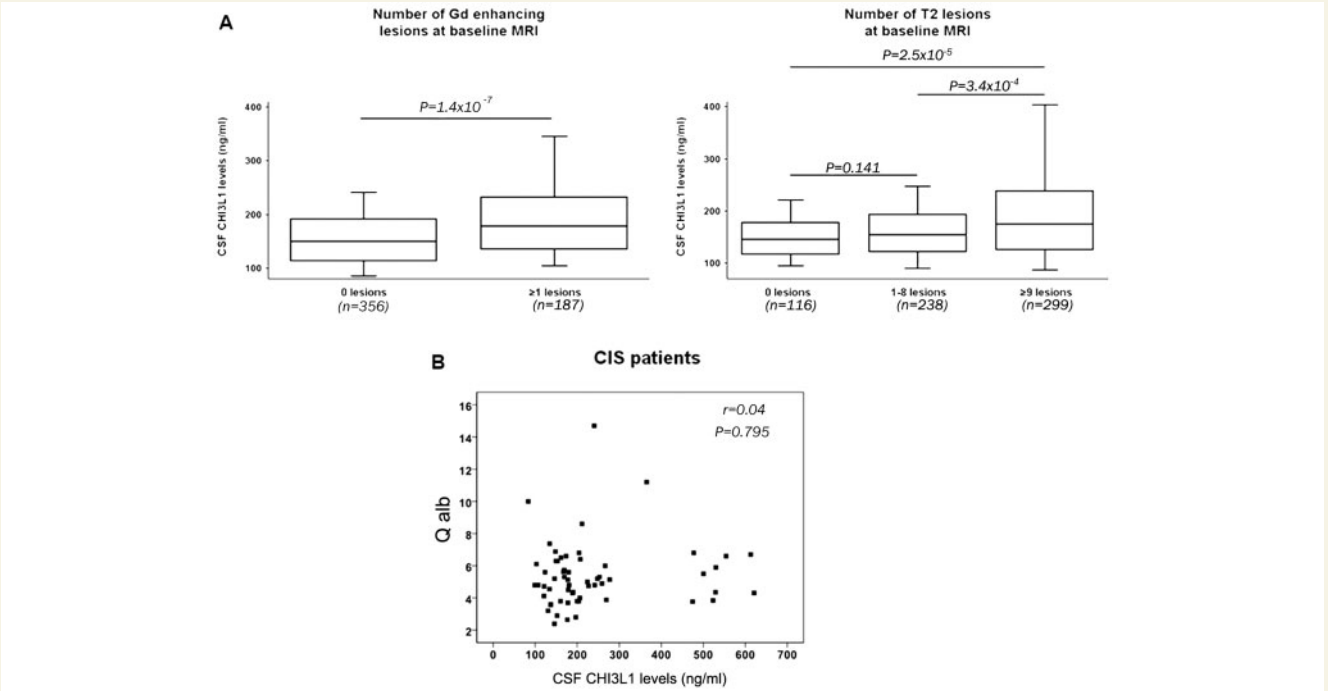


Figure 4 Association between CSF CHI3L1 levels and MRI abnormalities and CSF characteristics. (**A**) Boxplots showing age-adjusted CSF CHI3L1 levels in CIS patients stratified according to the presence of gadolinium (Gd) enhancing lesions (*left*) and number of T_2 lesions (*right*) at baseline. The number of Gd enhancing lesions was recoded into two categories: 0 lesions / 1 or more lesions. The number of T_2 lesions was recoded into three different categories: 0 lesions / 1–8 lesions / 9 or more lesions. Numbers in parentheses indicate individuals available for analysis. Analysis was performed with a Mann-Whitney's U-test. (**B**) Relationship between the albumin CSF/serum ratio (Q alb) and CHI3L1 protein levels in CSF. Albumin CSF/serum ratio was analysed in a subgroup of 59 CIS patients (40 patients from the University of Basel, Switzerland; and 19 patients from the University of Ulm, Germany). r = partial correlation coefficient.

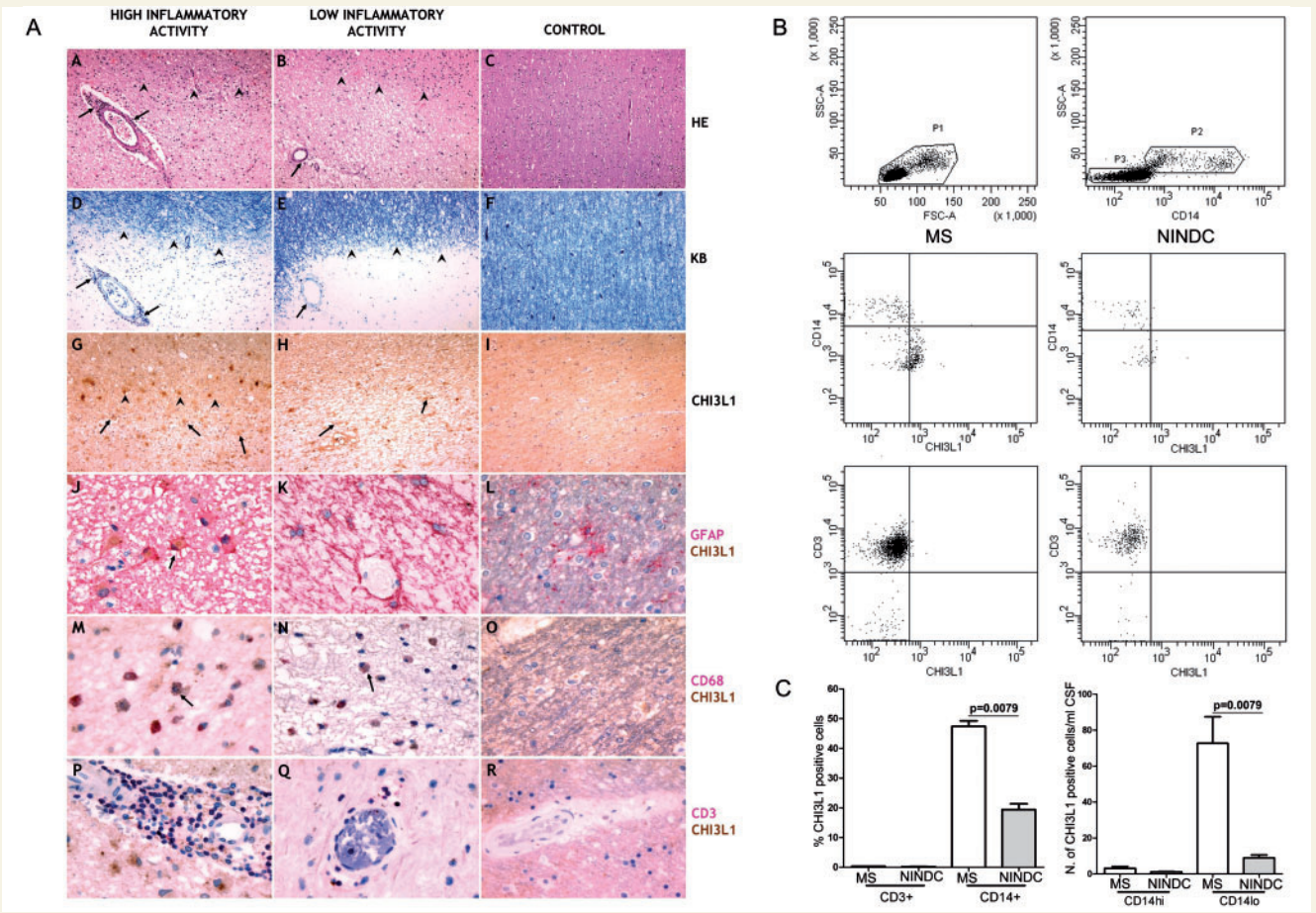


Figure 5 CHI3L1 expression in brain tissue and CSF cells. (**A**) CHI3L1 expression in chronic active lesions from multiple sclerosis patients and controls. Sections were stained with haematoxylin and eosin (HE; A–C) and Klüver-Barrera (KB; D–F), and subsequently classified into lesions with high inflammatory activity (A and D) and low inflammatory activity (B and E). Arrowheads indicate inflammatory infiltration observed at the edge of the lesions and arrows show perivascular inflammatory infiltration. Control samples did not show inflammatory cells or demyelination (C and F). CHI3L1 expression was observed at the edge of multiple sclerosis lesions (arrowheads) and in the demyelinated area (arrows) (G and H) but not in control samples (I). CHI3L1 expression was present in the cytoplasm of astrocytes (GFAP+) only in high inflammatory activity lesions (J–L), while it was observed within macrophages/microglial cells (CD68+ cells) in both types of lesions (M–O). T lymphocytes (CD3+) did not show CHI3L1 expression (P–R). (**B**) CHI3L1 expression in CSF cells from multiple sclerosis patients ($n = 5$) and non-inflammatory controls ($n = 5$; optic neuropathy, head trauma, arachnoid cyst, and two controls with neuropathies). *Top*: Representative dot plots showing gating strategy. An initial region (P1) was set on the forward/side scatter dot plot to include mononuclear cells and exclude debris or apoptotic cells. A second region was set around cells expressing intermediate to high CD14 with intermediate side scatter (monocytes, P2), and a third one around cells negative for CD14 with low side scatter (lymphocytes, P3). *Middle* and *bottom*: Representative dot plots showing intracellular CHI3L1 expression in monocytes (CD14+ cells) and T cells (CD3+ cells) respectively from a multiple sclerosis patient (*left*) and a control (*right*). (**C**, *left*) Bar graph showing the percentage of monocytes (CD14+ cells) and T cells (CD3+ cells) expressing CHI3L1 in multiple sclerosis patients (MS, $n = 5$) and non-INDC (NINDC, $n = 5$). *Right*: Bar graph showing CHI3L1 expression in monocytes classified according to CD14 expression: CD14 high ($CD14_{high}$) and CD14 low ($CD14_{low}$). A Mann-Whitney U-test was used to evaluate significant differences in CHI3L1 expression by CD3+ T cells and CD14+ monocytes between multiple sclerosis patients and controls.

persistently negative for CHI3L1 immunostaining (Fig. 5A, P and Q).

When CHI3L1 expression was explored by flow cytometry in CSF cells, protein expression was restricted to monocytes (CD14+ cells) from patients with multiple sclerosis and non-inflammatory controls, being higher in the multiple sclerosis group ($P = 0.008$ versus non-INDC; Fig. 5B and C). In contrast, CHI3L1 expression was absent in T cells (CD3+ cells) from multiple sclerosis patients and controls (Fig. 5B and C). Interestingly, monocytes with low expression of CD14 ($CD14_{low}$) were the major CSF cell subset expressing CHI3L1, particularly in the multiple sclerosis group ($P = 0.008$ when compared with non-INDC; Fig. 5C).

Discussion

CHI3L1 [also known as YKL-40, human cartilage glycoprotein 39 (HC-gp39), breast regression protein 39

(BRP-39)], is a member of the family of chitinases and chitinase-like proteins containing a highly conserved glyco-18 domain as common feature (Kzhyshkowska *et al.*, 2007). For these proteins, chitin is the only documented substrate (Kzhyshkowska *et al.*, 2007). However, whereas other chitinases of the family such as chitotriosidase and acidic mammalian chitinase AMCase have demonstrated chitinolytic activity (Renkema *et al.*, 1995; Boot *et al.*, 2001), CHI3L1 can bind chitin but lacks chitinolytic activity (Hakala *et al.*, 1993).

CHI3L1 is expressed by different cell types, including chondrocytes (Hakala *et al.*, 1993), vascular smooth muscle cells (Shackelton *et al.*, 1995), airway epithelial cells (Park *et al.*, 2010), neutrophils (Volck *et al.*, 1998), and macrophages (Renkema *et al.*, 1998). In the CNS, CHI3L1 expression has been mainly observed in astrocytes of monkeys and humans with lentiviral encephalitis (Bonneh-Barkay *et al.*, 2008), and patients with brain infarct (Bonneh-Barkay *et al.*, 2010).

Increased circulating levels of CHI3L1 have been reported in a wide variety of heterogeneous conditions characterized by chronic inflammation such as rheumatoid arthritis (Peltomaa *et al.*, 2001), osteoarthritis (Vos *et al.*, 2000), inflammatory bowel disease (Vind *et al.*, 2003), systemic lupus erythematosus (Vos *et al.*, 2000), asthma (Chupp *et al.*, 2007), sarcoidosis (Johansen *et al.*, 2005), hepatic fibrosis (Johansen *et al.*, 2000), atherosclerosis (Michelsen *et al.*, 2010), type 2 diabetes (Persson *et al.*, 2012), and obesity (Hempen *et al.*, 2009); in many instances, serum or plasma CHI3L1 levels correlated with disease activity and severity (Johansen *et al.*, 2000, 2005; Vos *et al.*, 2000; Peltomaa *et al.*, 2001; Vind *et al.*, 2003; Chupp *et al.*, 2007; Persson *et al.*, 2012). Circulating levels of CHI3L1 have also been found to be elevated in an extensive range of primary and metastatic cancers, including tumours of the ovary (Dupont *et al.*, 2004), endometrium (Peng *et al.*, 2010), breast (Jensen *et al.*, 2003), prostate (Brasso *et al.*, 2006), stomach (Bi *et al.*, 2009), colon/rectum (Cintin *et al.*, 1999), lung (Thom *et al.*, 2010), and brain (Iwamoto *et al.*, 2011), in which CHI3L1 levels were associated with poorer survival of cancer patients.

Regarding CNS disorders, serum levels of CHI3L1 were found to be increased in patients with stroke and correlated with functional outcome (Park *et al.*, 2012); plasma and CSF CHI3L1 levels were reported to have diagnostic implications in patients with preclinical and early Alzheimer's disease (Craig-Schapiro *et al.*, 2010; Choi *et al.*, 2011; Perrin *et al.*, 2011); CSF CHI3L1 levels were found to be elevated in patients with relapsing-remitting multiple sclerosis (Correale and Fiol, 2011), and plasma CHI3L1 levels were associated with the progressive forms of multiple sclerosis (Canto *et al.*, 2012).

Despite the numerous studies showing an implication of CHI3L1 in numerous disorders, its mechanism of action remains poorly understood, beyond few studies suggesting a function of CHI3L1 as tissue remodelling factor (Rehli *et al.*, 1997; Badariotti *et al.*, 2006; Bigg *et al.*, 2006). However, irrespective of this ignorance in its biophysiological activity, the abovementioned studies clearly point to a role of CHI3L1 as prognostic biomarker in a wide variety of conditions.

The potential role of CHI3L1 as prognostic biomarker in patients with CIS emerged from a screening proteomic study in a Spanish cohort in which CSF CHI3L1 levels were found to be elevated in patients who converted to clinically definite multiple sclerosis compared to those who continued as CIS (Comabella *et al.*, 2010). These initial findings have now been validated in a much larger cohort of CIS patients from different European multiple sclerosis centres, and CSF CHI3L1 levels were found again to be increased in CIS patients who later converted to clinically definite multiple sclerosis. The highest CSF levels of CHI3L1 were, however, observed in inflammatory controls, a finding somehow expected considering that a large proportion of these controls were suffering from CNS infectious diseases such as meningitis and encephalitis, conditions associated with extremely elevated CSF CHI3L1 levels (Ostergaard *et al.*, 2002). When the role of CHI3L1 as biomarker associated with the conversion to multiple sclerosis was further explored in multivariable Cox regression models, CSF CHI3L1 levels resulted in a risk factor for conversion to multiple sclerosis independent of strong predictors of conversion to multiple sclerosis such as brain MRI abnormalities and IgG oligoclonal bands (Tintore *et al.*, 2006, 2008; Fisniku *et al.*, 2008).

The long-term follow-up of some CIS patients included in the study allowed us to explore the role of CHI3L1 in the development of disability by examining the time to reach an EDSS score of 3.0. It is important to highlight that CSF CHI3L1 levels were a strong predictor of disability progression and, in fact, they were the only significant independent risk factor associated with the development of disability in multivariate Cox regression models.

Over 40% of patients with CIS had CSF CHI3L1 levels >170 ng/ml, a cut-off point with clear prognostic implications insomuch as patients with CHI3L1 levels above the cut-off value had earlier conversion to multiple sclerosis and earlier disability progression. This cut-off value of CSF CHI3L1 levels can be applied to clinical settings to identify those CIS patients with worse disease prognosis. The finding of high CSF CHI3L1 values, together with information from other variables such as the presence of IgG oligoclonal bands or three of four Barkhof criteria in the baseline MRI may aid the neurologist in the clinical decision-making of initiating treatment. While the behaviour of CHI3L1 as biomarker associated with conversion to multiple sclerosis is probably modest and not much different from other study variables, its role as prognostic biomarker is particularly relevant when the time to reach EDSS 3.0 is analysed, insomuch as CSF CHI3L1 levels above the 170 ng/ml cut-off were conferring, as a unique predictor, a 4-fold increased risk for the development of disability. Moreover, high CSF CHI3L1 levels were associated with

earlier disability progression (5-year difference as median time to reach EDSS 3.0 compared to patients with low protein values) with sensitivity over 70%. Based on this, CIS patients with high CSF CHI3L1 levels at the baseline lumbar puncture may benefit from early treatment to delay future development of disability.

The association between CSF CHI3L1 levels and both MRI abnormalities and CSF parameters at the time of the CIS event point to a close relationship between CHI3L1 levels and the degree of inflammation in the CNS of CIS patients. This relationship probably also explains the significant correlations observed between CHI3L1 levels and brain atrophy during follow-up; however, given the relatively small number of patients with brain atrophy data in the study, the role of CHI3L1 in the development of brain atrophy needs to be confirmed in larger cohorts of CIS patients and at longer time points.

The abovementioned association between CHI3L1 levels and CNS inflammation may prompt one to speculate that the blood-borne CNS inflammatory cell infiltration is the most likely source of CSF CHI3L1 levels. However, both albumin CSF/serum ratio data and immunohistochemistry findings argued against this hypothesis and suggest a primarily local CNS origin of CSF CHI3L1 levels. First, the independent behaviour of CSF CHI3L1 levels from the albumin CSF/serum ratio, a well-established measure for blood-CSF barrier dysfunction (Brettschneider *et al.*, 2005), indicates that CSF CHI3L1 protein is mainly brain derived. This notion is further supported by the 6-fold difference observed in CHI3L1 protein levels between CSF and serum samples (Comabella *et al.*, 2010). In consequence, CSF levels of CHI3L1 do not require any correction for blood concentration or albumin CSF/serum ratio, while necessary for blood-derived proteins such as IgG (Link and Tibbling, 1977). Second, CHI3L1 expression in multiple sclerosis chronic active lesions with high inflammatory activity largely derived from astrocytes, a finding already reported in a previous study (Bonneh-Barkay *et al.*, 2010). A contribution of blood-derived cells to CSF CHI3L1 levels cannot be ruled out based on the finding of CHI3L1 expression by CD68 positive macrophages/microglial cells in brain tissue and CD14 positive monocytes in CSF. However, the fact that CHI3L1 expression was mainly observed in CSF $CD14_{low}$ monocytes, a cell population reported to have decreased migratory potential to inflamed tissues due to the lack of chemokine receptor CCR5 (Geissmann *et al.*, 2003; Sunderkotter *et al.*, 2004; Louboutin *et al.*, 2011; Rossol *et al.*, 2012), suggests that CHI3L1 expression by $CD14_{low}$ monocytes is possibly not related to the blood-derived CNS inflammatory cell infiltration and these cells may be playing more critical roles in replacing resident macrophages (Geissmann *et al.*, 2003).

It can be hypothesized that brain macrophages/microglial cells and CSF $CD14_{low}$ monocytes are responsible for a low-level basal expression of CHI3L1 in the CNS, which can be further increased in proportion to the CNS inflammatory insult by the contribution of additional cells, primarily astrocytes. In these cases, the higher CSF CHI3L1 levels would reflect the degree of astrocyte activation secondary to inflammation. This is supported by the finding of CHI3L1 expression by reactive astrocytes in brain lesions with high inflammatory activity, and the positive correlations observed between CSF CHI3L1 levels and MRI abnormalities and inflammatory CSF parameters. The fact that high CSF CHI3L1 levels are associated with worse prognosis of CIS patients indirectly suggests a detrimental role of astrocyte activation in multiple sclerosis pathogenesis.

In conclusion, the aggregate results from this study confirm the prognostic role of CHI3L1 as biomarker associated with the conversion to multiple sclerosis and the development of disability in CIS patients. We propose to measure CSF CHI3L1 levels in CIS patients to identify those individuals with worse disease prognosis. The study also becomes the first example of large-scale validation of a CSF candidate biomarker in the field of multiple sclerosis.

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Supplementary material

Supplementary material is available at *Brain* online.

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