


BRIEF COMMUNICATION

Chitotriosidase is a biomarker for adult-onset leukoencephalopathy with axonal spheroids and pigmented glia

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Introduction

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), also known as *CSF1R-related leukoencephalopathy* or *Hereditary diffuse leukoencephalopathy with axonal spheroids* (HDLS), is a devastating disease characterised by rapidly progressive cognitive decline and motor impairment. First symptoms typically evolve in the fourth or fifth decade and include memory deficits, neuropsychiatric symptoms, and motor involvement.^{1–3} Patients are often demented and wheelchair-bound as early as 3 years after disease onset, and mean survival is 7 years.²

ALSP is an autosomal-dominant genetic disorder, implying a 50% risk of inheriting the disease for all first degree relatives. In 2012, Rademakers et al. identified the responsible gene, *Colony-stimulating factor 1 receptor* (*CSF1R*).⁴ *CSF1R* is a receptor-tyrosine kinase mainly found on cells of the myeloid lineage, including monocytes and macrophages.⁵ In the central nervous system, the principal cell

Abstract

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) leads to rapidly progressive dementia and is caused by mutations in the gene *CSF1R*. Neurodegeneration is driven by dysfunction of microglia, the predominant cell type expressing *CSF1R* in the brain. We assessed chitotriosidase, an enzyme secreted by microglia, in serum and cerebrospinal fluid of patients with ALSP. Chitotriosidase activity was highly increased in cerebrospinal fluid of patients and correlated inversely with disease duration. Of interest, presymptomatic *CSF1R* mutation carriers did not show elevated chitotriosidase levels. This makes chitotriosidase a promising new biomarker of disease activity for this rare disease.

type expressing *CSF1R* is microglia, the tissue-resident macrophages of the brain. The mechanisms leading from *CSF1R* mutation to manifest disease still remain unknown. However, microglia survival, proliferation, differentiation, and migration are dependent on *CSF1R* signalling,⁶ suggesting that impaired microglia function is responsible for the pathological changes occurring in ALSP.

In the present study we investigated chitotriosidase, an enzyme that is secreted by microglia, in ALSP and analysed chitotriosidase activity in correlation with cognitive function, clinical disability, and disease duration.

Methods

Patients and sample acquisition

Fourteen patients with genetically confirmed ALSP (median age 48 years, range 33–59) and seven presymptomatic *CSF1R* mutation carriers (median age 30 years,

range 25–73) were recruited at the leukodystrophy outpatient clinic in Tübingen (Germany) (Table S1). All probands underwent standardised neurological examination. Activities of daily living were assessed using the Barthel index,⁷ and cognitive status was screened by Montreal Cognitive Assessment (MoCA).⁸ Serum and CSF were obtained according to standard procedures and stored at -80°C until further processing. Disease duration was defined as the period from first reported symptom to sampling. Control serum and CSF of 10 healthy controls without symptoms of neurological disease (median age 49 years, range 18–81) was obtained from the Biobank of the Hertie Institute for Clinical Brain Research (HIH), University of Tübingen. The study has been evaluated by the local Institutional Review Board (Vote 690/2011BO1). All participants gave their written informed consent prior to inclusion.

Chitotriosidase measurements

Chitotriosidase was measured according to Hollak et al.⁹ In brief, 5 μL CSF or serum were incubated with 50 μL of 0.022 mmol/L 4-Methylumbelliferyl- β -D-N,N',N''-triacetyl-chitotriosidase-citrate-phosphate (0.1/0.2 mol/L)-buffer (Merck, Darmstadt, Germany), pH 5.2, for 15 min at 37°C and attenuated with 1 mL 20% alkaline buffer solution (1.5 mol/L, pH 10.3; Merck, Darmstadt, Germany) in water. The cleavage product 4-methylumbelliferone was measured fluorometrically at 460 nm (excitation at 355 nm). Triplicate measurements were performed for all samples except for CSF of one patient, where CSF volume was only available for two measurements.

Statistical analysis

Statistical significance was assessed using GraphPad Prism Version 9.3.0. Gaussian distribution was tested using the D'Agostino-Pearson omnibus normality test. Depending on the type of distribution, differences between groups were analysed using a parametric or non-parametric unpaired two-tailed *t*-test and one-way ANOVA with Tukey's multiple comparison test, respectively. Likewise, correlation was analysed using either Pearson *r* for normally distributed data or Spearman *r* for data that did not pass the normality test. The applied test is indicated in the text and figure legend, respectively. Numerical results represent mean \pm standard error of the mean, graphical results mean \pm standard deviation.

Results

Serum and CSF were available from 14 patients with ALSP. In addition, serum of 7 presymptomatic carriers of *CSF1R* mutations has been included. Given the known influence of ageing on chitotriosidase activity,^{10,11} we first performed a correlation analysis of serum as well as CSF with age in all groups. In neither group, chitotriosidase activity correlated with age (Fig. S1).

Serum chitotriosidase activity was elevated in patients with ALSP compared to healthy controls (controls: 28.7 ± 3.3 nmol/h/mL, 95% confidence interval (CI): 21.6 to 36.0, $n = 10$; ALSP: 64.7 ± 11.3 nmol/h/mL, 95% CI: 40.3 to 89.2, $n = 14$, $p = 0.016$ (unpaired *t*-test)) (Fig. 1A). Similarly, in CSF we found chitotriosidase activity to be highly increased in patients with ALSP (controls:

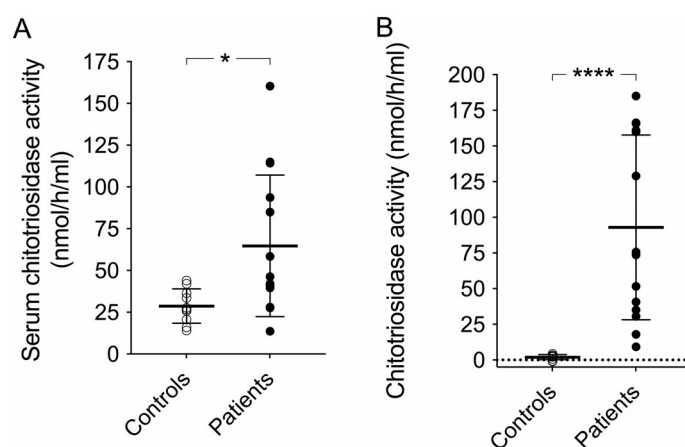


Figure 1. Chitotriosidase activity is increased in ALSP. (A) Chitotriosidase activity in serum of patients with ALSP compared to controls; $*p = 0.016$ (unpaired *t*-test). (B) Chitotriosidase activity in CSF of patients with ALSP compared to controls. $****p < 0.0001$ (Mann–Whitney test). CSF, cerebrospinal fluid.

2.0 ± 0.6 nmol/h/mL, 95% CI: 0.7 to 3.2, $n = 10$; ALSP: 93.0 ± 17.3 nmol/h/mL, 95% CI: 55.6 to 130.4, $n = 14$, $p < 0.0001$ (Mann–Whitney test)) (Fig. 1B).

Next, we investigated clinical determinants of chitotriosidase activity. Chitotriosidase activity did not correlate with cognitive function (tested by MoCA) or clinical disability (assessed by Barthel Index), neither in serum nor in CSF (Fig. 2A and B). However, there was a strong negative association between chitotriosidase activity in CSF and disease duration in patients with ALSP: the shorter the disease duration, the higher was the chitotriosidase activity ($r^2 = 0.410$, $n = 13$, $p = 0.018$ (Pearson r)) (Fig. 2C, right panel). This correlation was not found in serum (Fig. 2C, left panel).

When examining the correlation of chitotriosidase activity between serum and CSF of patients with ALSP, we found a moderate positive association ($r^2 = 0.289$, $n = 14$, $p = 0.049$ (Spearman r)) (Fig. 3A). As we have recently identified neurofilament light chain (NfL) in serum and CSF as a biomarker in ALSP,¹² we investigated the correlation between these biomarkers. We found a strong correlation between NfL and chitotriosidase in CSF, but not in serum (serum: $r^2 = 0.019$, $n = 10$, $p = 0.707$ (Spearman r); CSF: $r^2 = 0.614$, $n = 11$, $p = 0.017$ (Spearman r)) (Fig. 3B and C).

We further analysed chitotriosidase activity in presymptomatic *CSF1R* mutation carriers. As there was no CSF available from presymptomatic carriers, we compared serum chitotriosidase activity between carriers and healthy controls as well as patients with ALSP. The levels in carriers did not differ from those of controls, but from those of patients (controls: 28.7 ± 3.3 nmol/h/mL, 95% confidence interval: 21.6 to 36.0, $n = 10$; mutation carriers: 24.0 ± 3.2 nmol/h/mL, 95% CI: 16.2 to 31.8, $n = 7$; ALSP: 64.7 ± 11.3 nmol/h/mL, 95% CI: 40.3 to 89.2, $n = 14$; controls vs. mutation carriers: $p = 0.946$; mutation carriers vs. ALSP: $p = 0.017$ (one-way ANOVA with Tukey's multiple comparison test)) (Fig. 4).

Discussion

We found chitotriosidase activity to be highly increased in serum and CSF of patients with ALSP. CSF levels differentiated perfectly between patients and controls. In addition, we found an inverse correlation of chitotriosidase activity with disease duration – the shorter the disease duration, the higher the activity. In contrast, chitotriosidase was not increased in serum of presymptomatic *CSF1R* mutation carriers.

The physiological role of chitotriosidase in humans is not entirely resolved. Chitotriosidase was shown to be mainly expressed in innate immune cells such as activated macrophages and microglia,^{9,13,14} and that pro-

inflammatory stimuli elicit chitotriosidase secretion.¹⁵ In ALSP, chitotriosidase elevation may represent an activation of either microglia or infiltrating macrophages, but most likely is the common final pathway of both. Independent of the source, the strong elevation of chitotriosidase in the CNS compartment could reflect an inflammatory state of brains affected by ALSP. In this context, chitotriosidase may not necessarily have a detrimental influence on CNS tissue, but likely represents an epiphenomenon occurring in disease pathophysiology, or even has a protective role. Whether the putative inflammation is of primary cause, i.e. autoimmune, or secondary, for example due to excessive cell debris, remains to be resolved. Independent of the aetiology, our finding of an inverse correlation of chitotriosidase activity with disease duration and of normal levels in presymptomatic *CSF1R* mutation carriers suggests a dynamic process. Longitudinal assessment is required to demonstrate the evolution of chitotriosidase activity; our pilot data suggest a decrease over time, potentially caused by a progressive loss of functional microglia during the course of the disease.

According to our data, chitotriosidase may also help in monitoring the conversion from presymptomatic state to manifest disease in *CSF1R* mutation carriers. This is of special interest to determine the optimal time point for haematopoietic stem cell transplantation as the only available treatment in ALSP.¹⁶ Longitudinal assessment of *CSF1R* mutation carriers covering the period around disease manifestation is required to prove the value of chitotriosidase activity as marker of imminent conversion into manifest disease.

Chitotriosidase is also found elevated in other neurological diseases such as Gaucher disease, amyotrophic lateral sclerosis, multiple sclerosis, and Alzheimer's disease.^{9,11,17–19} These diseases are clinically distinct from ALSP in terms of progression and symptoms, and/or show different imaging features. Highly elevated chitotriosidase in a patient with clinical and imaging features of ALSP is therefore a valuable marker to support the diagnosis of ALSP and should lead to the initiation of genetic testing. In addition, although the diagnosis of ALSP is primarily made by genetic testing, diagnostic uncertainty may arise with novel variants of unknown significance in the *CSF1R* gene. In such cases, the analysis of chitotriosidase helps to confirm the pathogenic character of a variant. Nonetheless, before chitotriosidase can be established as a distinct diagnostic biomarker for ALSP, it needs to be evaluated in larger cohorts, and other leukoencephalopathies with similar clinical presentation. It also has to be noted that chitotriosidase deficiency can be found in 6% of healthy individuals.^{9,18–20}

Taken together, we propose chitotriosidase as a new biomarker in ALSP, substantiating the notion that

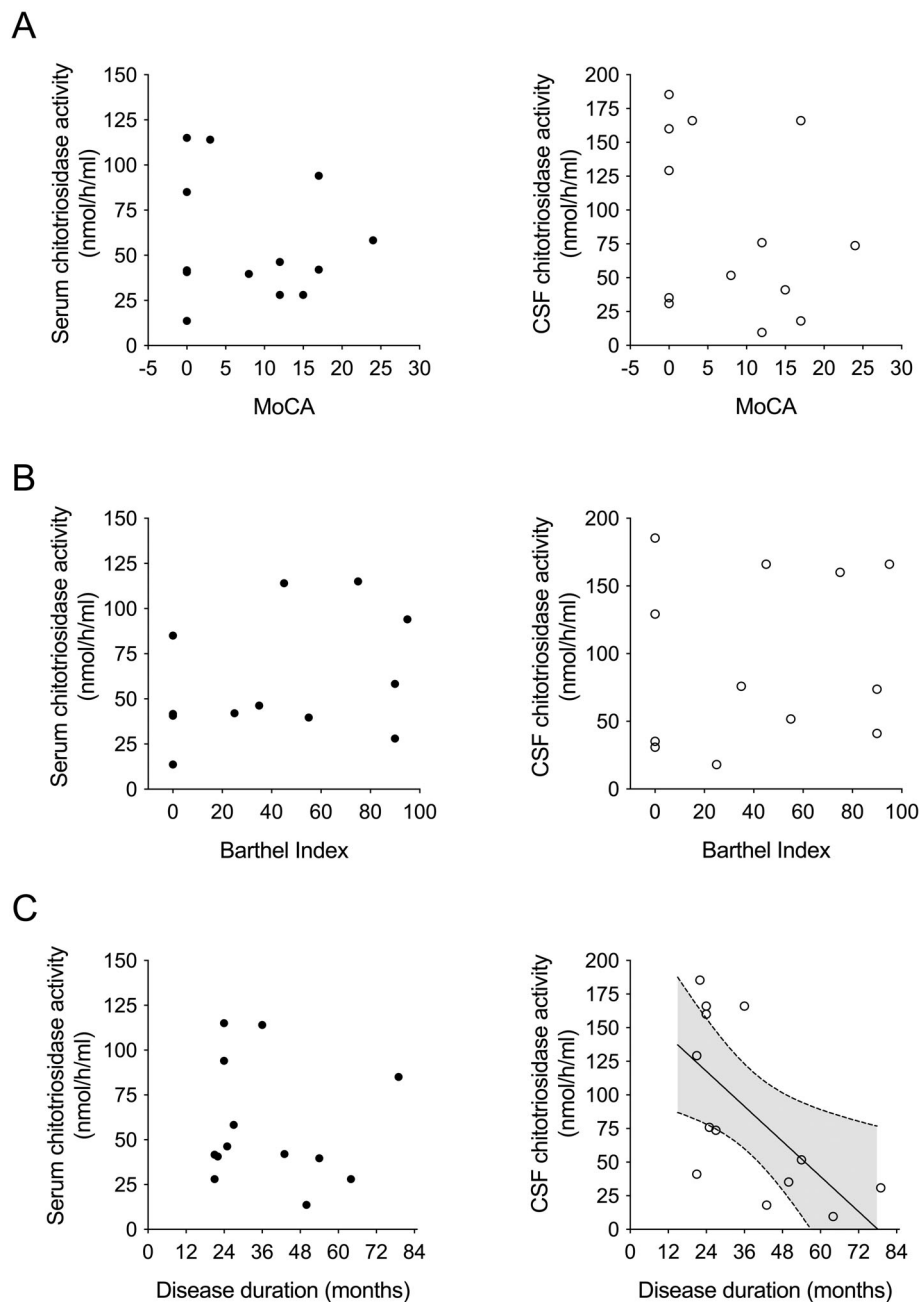


Figure 2. Chitotriosidase activity correlates inversely with disease duration in ALSP. (A) Chitotriosidase activity in serum and CSF does not correlate with MoCA score of patients with ALSP. Serum (left panel): $r^2 = 0.024$, $n = 13$, $p = 0.616$ (Pearson r); CSF (right panel): $r^2 = 0.1$, $n = 13$, $p = 0.293$ (Pearson r). (B) Chitotriosidase activity in serum and CSF does not correlate with Barthel Index of patients with ALSP. Serum (left panel): $r^2 = 0.113$, $n = 12$, $p = 0.285$ (Pearson r); CSF (right panel): $r^2 = 0.016$, $n = 12$, $p = 0.692$ (Pearson r). (C) Chitotriosidase activity correlates inversely with disease duration in CSF, but not in serum. Serum (left panel): $r^2 = 0.011$, $n = 13$, $p = 0.739$ (Pearson r); CSF (right panel): $r^2 = 0.410$, $n = 13$, $p = 0.018$ (Pearson r), simple linear regression with dashed lines representing SEM. CSF, cerebrospinal fluid. MoCA, Montreal Cognitive Assessment.

microglia play an important role in this devastating disease, even though its contribution – beneficial or detrimental – remains to be elucidated. It will be intriguing to

analyse larger cohorts, longitudinal measurements, and transplanted patients to reveal the applicability of chitotriosidase activity as a biomarker in ALSP.

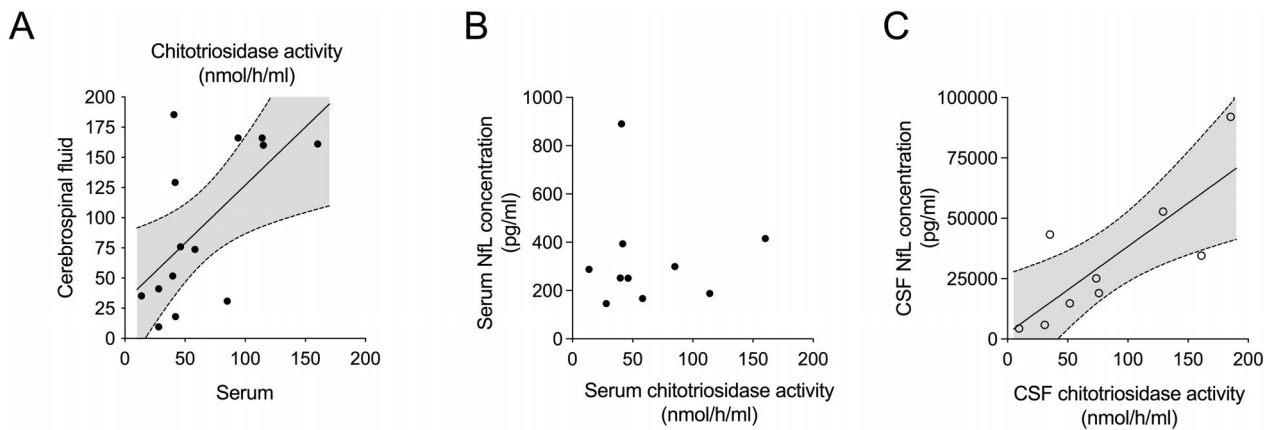


Figure 3. Chitotriosidase activity and NfL in patients with ALSP. (A) The activity of serum chitotriosidase moderately correlates with the activity in CSF of patients with ALSP. (B) Chitotriosidase activity in serum does not correlate with serum NfL in patients with ALSP. (C) CSF chitotriosidase activity and CSF NfL strongly correlate in patients with ALSP. CSF, cerebrospinal fluid. NfL, Neurofilament light chain.

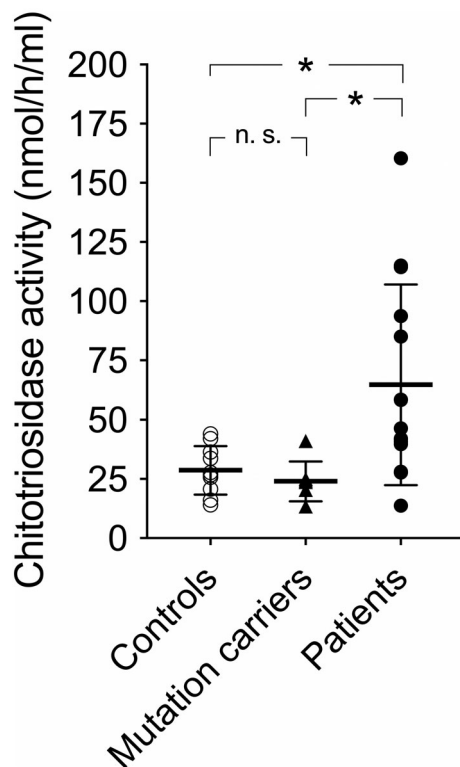


Figure 4. Chitotriosidase activity in presymptomatic *CSF1R* mutation carriers. Serum chitotriosidase activity in *CSF1R* mutation carriers is similar to controls, and significantly lower than in patients with ALSP. * $p < 0.05$. n. s., not significant.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Stefanie N. Hayer: Study concept and design, patient care, acquisition of biomaterials, data analysis, drafting and revision of the manuscript.

Vidiyaah Santhanakumaran: Chitotriosidase measurements, revision of the manuscript.

Judith Böhringer: Chitotriosidase measurements, revision of the manuscript.

Ludger Schöls: Study concept and design, patient care, acquisition of biomaterials, revision of the manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Correlation analysis of chitotriosidase activity with age. Chitotriosidase activity in serum/CSF did not correlate with age in the groups tested. Controls: $r^2 = 0.047$, $n = 10$, $p = 0.547$ (Pearson r); presymptomatic CSF1R mutation carriers: $r^2 = 0.040$, $n = 7$, $p = 0.676$ (Spearman r); ALSP: serum: $r^2 = 0.241$, $n = 14$, $p = 0.074$ (Pearson r), CSF: $r^2 = 0.120$, $n = 14$, $p = 0.222$ (Spearman r). CSF, cerebrospinal fluid.

Table S1 CSF1R mutations and age at sample acquisition.