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Institutions: University of Ulm, Umeå University, Technische Universität München, University of Erlangen-Nuremberg ...+6 more institutions

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Research article

Different neuroinflammatory profile in ALS and FTD is linked to the clinical phase

Patrick Oeckl PhD[1], Patrick Weydt MD[1§], Petra Steinacker PhD[1], Sarah Anderl-Straub PhD[1], Frida Nordin MD[2], Alexander E. Volk MD[3], Janine Diehl-Schmid MD[4], Peter M. Andersen MD, PhD[1,2], Johannes Kornhuber MD[5], Adrian Danek MD[6], Klaus Fassbender MD[7], Klaus Fliessbach MD[8], German Consortium for Frontotemporal Lobar Degeneration[9], Holger Jahn MD[10], Martin Lauer MD[11], Kathrin Müller PhD[1], Antje Knehr[1], Johannes Prudlo MD[12], Anja Schneider MD[8], Dietmar R. Thal MD[13,14], Deniz Yilmazer-Hanke MD[1], Jochen H. Weishaupt MD[1], Albert C. Ludolph MD[1], Markus Otto MD[1]

[1]Department of Neurology, Ulm University Hospital, Ulm, Germany

[2]Department of Pharmacology and Clinical Neurosciences, Umeå University, Umeå, Sweden.

[3]Institute of Human Genetics, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

[4]Department of Psychiatry, Technical University of Munich, Munich, Germany

[5]Department of Psychiatry, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany

[6]Department of Neurology, LMU Munich, Munich, Germany

[7]Department of Neurology, Saarland University, Homburg, Germany

[8]Department of Neurodegenerative Diseases and Gerontopsychiatry, University of Bonn and DZNE Bonn, Bonn, Germany

[9]German Consortium for Frontotemporal Lobar Degeneration, University of Ulm, Ulm, Germany (www.ftld.de)

[10]Department of Psychiatry, University Hospital Hamburg, Germany

[11]Department of Psychiatry and Psychotherapy, University of Würzburg, Würzburg, Germany

[12]Department of Neurology, University of Rostock, and German Center for Neurodegenerative Diseases (DZNE), Rostock, Germany

[13]Department of Neuroscience, KU Leuven and Department of Pathology, UZ Leuven, Leuven, Belgium

[14]Laboratory of Neuropathology – Institute of Pathology, Ulm University, Ulm, Germany

§current address: Department of Neurodegenerative Diseases and Gerontopsychiatry, University of Bonn, Germany

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Corresponding author:
Prof. Dr. med. Markus Otto
Ulm University Hospital
Department of Neurology
Oberer Eselsberg 45
D-89081 Ulm
phone: +49-731-500-63010
fax: +49-731-500-63012
e-mail address: markus.otto@uni-ulm.de

Key Points

Question: Is neuroinflammation present in the presymptomatic phase of ALS and FTD and is there a different profile between ALS and FTD.

Findings: In this case-control study with ALS/FTD gene mutation carriers, CSF levels of neuroinflammatory markers (CHIT1, YKL-40, GFAP) were unchanged in asymptomatic mutation carriers. In contrast, levels were markedly increased in symptomatic ALS and FTD cases (genetic and sporadic) but with a different profile between ALS and FTD.

Meaning: Neuroinflammation is linked to the symptomatic phase of ALS/FTD and the different profile between ALS and FTD could be one driver of the diverse presentations of the ALS/FTD syndrome.

Abstract

Importance: Neuroinflammation plays a role in the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) but its contribution to the early disease phase, differences between sporadic (sALS, sFTD) and genetic (gALS, gFTD) cases, or between ALS and FTD is unclear and data mainly based on non-human disease models.

Objective: To investigate the role of neuroinflammation in asymptomatic and symptomatic ALS and FTD mutation carriers.

Design: In this case-control study, individuals were recruited during 2011-2017 (Ulm, German Presymptomatic ALS study, German FTLD consortium) and 1987-2012 (Umeå).

Setting: Multicenter study

Participants: We investigated asymptomatic ALS/FTD mutation carriers (n=16), gALS (n=65), gFTD (n=23), sALS (n=64/70), and sFTD patients (n=20/26) and control patients without neurodegenerative diseases (n=36/32). Asymptomatic ALS/FTD mutation carriers were first-degree relatives of gALS patients.

Main Measures: The neuroinflammatory markers chitotriosidase 1 (CHIT1), YKL-40, and GFAP were measured in CSF and blood.

Results: CSF levels of CHIT1, YKL-40, and GFAP were unaffected in asymptomatic mutation carriers. CHIT1 and YKL-40 were increased in gALS whereas GFAP was not affected. ALS patients carrying a *CHIT1* polymorphism had lower CHIT1 concentrations in CSF (-80%) whereas this polymorphism had no influence on neurofilament levels and age at disease onset. In gFTD, increased YKL-40 and GFAP was observed, whereas CHIT1 was nearly not affected. This could be confirmed in *post-mortem* spinal cord tissue. The same profile was observed in sALS and sFTD. GFAP showed a sensitivity and specificity of 75% and 83% to discriminate FTD from ALS.

Conclusions: Our data indicate that neuroinflammation is linked to the symptomatic phase of ALS/FTD and shows a similar pattern in sporadic and genetic cases. ALS and FTD are characterized by a different neuroinflammatory profile, which might be one driver of the diverse presentations of the ALS/FTD syndrome and help in the differential diagnosis.

Keywords: Neuroinflammation, amyotrophic lateral sclerosis, frontotemporal dementia

Introduction

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are rare neurodegenerative diseases with a prevalence of 3-5/100,000 persons for ALS and 10-30/100,000 persons in the age of 45-65 years for FTD[1,2]. Owing to an overlap of neuropathological characteristics, clinical symptoms and disease-causing genes, both diseases are thought to represent different manifestations of a single disease syndrome with shared pathogenesis[2,3]. To date, the cause of ALS and FTD is unclear for most (i.e. sporadic) cases (sALS and sFTD) except for 5-10% of patients with a clear monogenic background (gALS, gFTD) and there is no disease-modifying treatment option available[4,5]. It is also elusive which factors determine the commitment to ALS versus FTD. The most prevalent identified gene mutation in both disease entities is a large intronic GGGGCC-hexanucleotide expansion in *C9orf72*. Other genes affected in gALS or gFTD include *SOD1*, *FUS*, *TARDBP*, *TBK1, NEK1*, *MAPT*, and *GRN*[4,6].

A major problem for studying early pathophysiological alterations in ALS and FTD is that their diagnosis relies on clinical symptoms[7,8] which is why the preclinical phase of the diseases is not accessible to researchers. Knowledge about pathophysiological processes in the presymptomatic phase is important for early diagnosis and the development of disease-modifying treatment strategies and is mainly derived from mutation-based animal models. In this context, we established a cohort of asymptomatic ALS- and FTD-gene mutation carriers to study the preclinical phase of ALS/FTD in human individuals. Using this cohort, we could successfully show that neurofilament (Nf) levels in cerebrospinal fluid (CSF), as markers of axonal degeneration, increase massively with disease onset[9]. Conversely, the *C9orf72*-related expression of dipeptide repeats (DPRs) is already observed in the asymptomatic phase of c9ALS/FTD but absent in sporadic cases[10]. This supports the use of asymptomatic gene mutation carriers for the study of the presymptomatic disease phase and also highlights the need for comparative studies between genetic and sporadic cases.

Neuroinflammation is an important hallmark of ALS and FTD and increased levels of neuroinflammatory markers in CSF and *post-mortem* autopsies as well as the observation of disease-causing mutations in inflammation-related genes (reviewed in[11,12]) suggest that it may contribute to neurodegeneration in ALS/FTD. However, its temporal role is unclear and relies largely on transgenic animal models[12] and a direct comparison of the neuroinflammatory pattern between ALS and FTD is also missing.

In the present study, we used the inflammatory marker proteins chitotriosidase 1 (CHIT1), chitinase-3-like protein 1 (CHI3L1, YKL-40), and glial fibrillary acidic protein (GFAP) in CSF to characterize neuroinflammation in asymptomatic and symptomatic ALS/FTD gene mutation carriers to get a hint about the temporal initiation of neuroinflammation in ALS/FTD. In addition, we compared the neuroinflammatory profile between ALS and FTD patients and also between sporadic and genetic cases.

Methods

Patients

The genetic patient cohort (table 1) consisted of gALS patients recruited at the Departments of Neurology of the Ulm University Hospital and University of Umeå and gFTD patients (all with the behavioural variant of FTD, bvFTD) enrolled at different clinical centers of the German FTLD consortium (Ulm, Munich, Erlangen, Homburg, Bonn, FTLDc-TRACE study). Asymptomatic first-degree relatives of familial ALS patients were recruited via the German Presymptomatic (GPS)-ALS cohort.[9] First degree relatives without a mutation were assigned to the control group, and mutation carriers without signs of upper or lower motor neuron affection formed the group of asymptomatic ALS/FTD mutation carriers.

Patients of the sporadic patient cohort (table 2), recruited at the Department of Neurology, Ulm University Hospital, included sALS, sFTD (all bvFTD), and control patients without neurodegenerative disease.

ALS and FTD patients were diagnosed according to accepted criteria.[7,8] All patients or their

relatives gave written informed consent. The Medical Ethical Review Boards of the participating centers approved the study.

All patients underwent neuropsychological testing using standard procedures. Disease severity in ALS patients was assessed using the ALSFRS-R (ALS Functional Rating Scale-revised) and in FTD patients using the FTLD-specific Clinical Dementia Rating (FTLD-CDR) score. Genetic testing for a panel of known ALS/FTD genes was performed according to standard protocols (details available upon request). The 24bp-duplication of *CHIT1* (c.1049_1072dup, NM_003465.2) was detected by size determination in agarose gel (4%) electrophoresis after PCR amplification.

CSF was collected at diagnostic evaluation by lumbar puncture (LP), centrifuged, and stored within 2h at -80°C. Plasma (Umea cohort only) and serum samples (herein after referred to as blood samples) were treated likewise.

Post-mortem spinal cord samples were obtained from five gALS patients (two female, three male, age: 52.8±12.6 years) and one gFTD patient (male, age 75 years) carrying *C9orf72* mutations and five neurological controls (two female, three male, age: 57.6±10.1 years) with the following diagnosis: (1) polyradiculits and toxic myopathy, (2) subdural hematoma, hemoragic infarct, and multiple sclerosis, (3) cerebral microangiopathy, (4) subdural hematoma, intracerebral bleeding, and small vessel disease, and (5) argyrophilic grain disease and subcortical vascular encephalopathy.

Biomarker determination in CSF, blood, and spinal cord tissue

CSF and blood concentrations of CHIT1 were measured using an ELISA from MBL (Belgium).[13] YKL-40 was measured with the MicroVue ELISA from Quidel (USA).[14] GFAP in CSF was determined with an ELISA from BioVendor (Czech Republic) and GFAP in blood was measured with the Simoa GFAP Discovery Kit (Quanterix, USA). Neurofilaments (neurofilament light chain, NfL, phosphorylated neurofilament heavy chain, pNfH) were measured using ELISAs from Uman Diagnostics, Sweden (NfL) and BioVendor, Czech

Republic (pNfH), respectively.

CHIT1 expression in post-mortem spinal cord tissue of five gALS and one gFTD patients (all with large hexanucleotide expansions in *C9orf72*), four non-neurodegenerative controls, and one multiple sclerosis patient was analyzed with immunoblot using a rabbit-anti-CHIT1 antibody (Sigma #HPA010575). GAPDH (glyceraldehyde 3-phosphate dehydrogenase) expression was used for normalization.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0. Groups were compared by Mann-Whitney test (two-tailed) or Kruskal-Wallis test and Dunn´s post-hoc test. Correlation analyses were performed using Spearman´s rank correlation coefficient. CSF YKL-40 and blood GFAP was age-adjusted using a linear regression model. Frequencies of the *CHIT1* 24bp-duplication in exon 10, their deviation from Hardy-Weinberg equilibrium and of sex in the cohorts were compared by Chi-square test. Densitometric analysis of immunoblots was performed using ImageJ 1.48v software, and CHIT1 expression in spinal cord tissue was normalized to GAPDH and compared by Student´s *t*-test (two-tailed). The discriminatory potential of YKL-40 and GFAP was determined using receiver operating characteristic (ROC) curve analysis, and cut-offs were calculated using the Youden index. A p-value <0.05 was regarded as statistically significant.

Results

CHIT1

Characteristics of patients are listed in table 1 (genetic ALS/FTD cohort) and 2 (sporadic ALS/FTD cohort). The gALS and gFTD patients were older than controls (and asymptomatic mutation carriers ($p<0.001$). The median CHIT1 concentration in CSF was four- to ninefold increased in the gALS patients compared to controls, asymptomatic mutation carriers, and gFTD cases. The other groups did not differ statistically from one another (Fig. 1A).

Correlation of CHIT1 concentration in CSF with age in controls was weak (r=0.37) and not significant (p=0.11). No significant differences of CHIT1 were observed in blood except between gALS from Ulm and controls (eFig. 1A). Blood and CSF concentrations of CHIT1 were significantly correlated (r=0.51, p<0.0001) (eFig. 1B) and CHIT1 concentrations in CSF were strongly positively correlated with concentrations of the axonal degeneration markers NfL and pNfH (p<0.0001, eFig. 1C). We confirmed increased CHIT1 expression also in spinal cord tissue of gALS cases by immunoblot (p<0.05) whereas no alteration was observed in the single autopsied FTD case (Fig. 1B,C).

A 24bp-duplication in exon 10 of the *CHIT1* gene has been described previously resulting in reduced expression and activity of CHIT1.[15] Because the prevalence of this polymorphism is high in European populations (35-50%)[16], we genotyped our genetic patient cohort to rule out differences in CHIT1 concentrations due to *CHIT1* genotypes. About 43% (n=12) and 4% (n=1) of control patients were either heterozygous or homozygous carriers of the 24bp-duplication of *CHIT1* (Fig. 1D), and the affected individuals are also highlighted in Fig. 1A and eFig. 1A. In all groups, no significant difference of the CHIT1 genotype frequency to the Hardy-Weinberg equilibrium was observed. There was a tendency towards a lower frequency of this *CHIT1* mutation in gALS cases from both centers, but this difference was not significant (p=0.38). CHIT1 concentrations were lower in CSF and serum in heterozygous and homozygous carriers of the 24bp duplication. Notably, CHIT1 concentrations were increased in CSF in gALS patients independently from the *CHIT1* genotype (Fig. 1E).

Genetic ALS patients carrying the *CHIT1* 24bp-duplication had significantly lower CHIT1 concentrations in CSF compared with non-carriers, but neurofilament concentrations and age at disease onset were not affected by the *CHIT1* polymorphism (Fig. 2F).

YKL-40

In agreement with previous studies[17,18], YKL-40 showed a moderate correlation with age in

CSF of control patients (r=0.41, p=0.01) but not in serum (r=0.17, p=0.38) therefore only CSF values of YKL-40 were age-adjusted. CSF YKL-40 was significantly increased in gALS and gFTD compared with controls and asymptomatic mutation carriers (Fig. 2A) by a factor of two to five. There was no difference between controls and asymptomatic mutation carriers and between gALS and gFTD. We did not observe alterations in blood YKL-40 (p=0.36, eFig. 2A) and there was only a weak correlation between CSF and blood concentrations (r=0.30, eFig. 2B).. Since data about YKL-40 in CSF of sALS patients is limited, we investigated an additional cohort of age- (p=0.88) and sex-matched (p=0.91) sALS and sFTD patients. Both sALS and sFTD patients had increased concentration of YKL-40 in CSF with slightly higher values in sFTD compared to sALS (p<0.05, Fig. 2B). Correlation analysis showed a strong correlation of CSF YKL-40 concentrations with the axonal degeneration marker NfL (r=0.73, p<0.0001, eFig. 2C) and pNfH (r=0.71, p<0.0001).

GFAP

The GFAP concentration in CSF was significantly increased in gFTD compared with controls (p<0.05) and gALS (p<0.05, Fig. 2C). There was no difference between controls, asymptomatic mutation carriers, and gALS groups. Blood concentrations of GFAP did not differ between the groups studied (eFig. 3A). However, blood concentrations showed a strong correlation with age in control samples (r=0.70, p<0·0001) and therefore they were age-adjusted. GFAP in CSF did not significantly correlate with age (r=0.28, p=0.05). We observed a weak correlation between blood and CSF GFAP (r=0.39, eFig. 3B). For comparison with the genetic cases, we also analyzed a larger group of age (p=0.88) and sex-matched (p=0.83) sALS and sFTD cases. Similarly, higher CSF GFAP concentrations were observed in sFTD patients compared with sALS (p<0.001) and controls (p<0.01, Fig. 2B). The correlation of CSF GFAP with NfL and pNfH in the whole genetic cohort was very weak (r=0.04 and r=0.05, eFig. 3C) and also weak in the gFTD group (r=0.32, p=0.34 and r=0.28, p=0.38).

ROC analysis and time course estimation

To evaluate the diagnostic potential of YKL-40 and GFAP a ROC analysis was performed including all patients from the genetic and sporadic cohorts. The area under the curve (AUC), sensitivity, and specificity and the corresponding cut-offs are given in Fig. 3A (YKL-40) and Fig. 3B (GFAP). Although we observed a strong increase of CSF CHIT1 in symptomatic ALS which makes it an interesting biomarker candidate e.g. in clinical trials with well-characterized patients, the high prevalence of the *CHIT1* polymorphism on exon 10 possibly makes CHIT1 unsuitable as a diagnostic biomarker in clinical practice. We thus decided that it is not useful to calculate ROC curves and a diagnostic threshold here. Clearly, the genetic *CHIT1* status must be taken into account when interpreting CHIT1 concentrations as a biomarker. Correlation analysis of the three inflammatory markers in CSF yielded a moderately strong correlation of CHIT1 with YKL-40 ($r=0.52$, $p<0.0001$) and very weak correlation of CHIT1 and YKL-40 with GFAP ($r=0.14$, $p=0.28$ and $r=0.09$, $p=0.46$).

To simulate a presymptomatic time span, we used the parental age of onset to estimate the time to disease onset as previously described[9]. The estimated time course of CHIT1, YKL-40 and NfL is shown in Fig. 3C for the asymptomatic mutation carriers and gALS cases. Here, a sudden increase of CHIT1 and YKL-40 with symptom onset, similar to the previously reported neurofilaments[9], is seen.

Discussion

The identification of early pathophysiological events in ALS and FTD is challenging because diagnosis is based on clinical symptoms[7,8] making presymptomatic disease phases inaccessible to researchers. Studies of ALS/FTD mutation carriers offer a unique opportunity to investigate the critical preclinical phase because they can be identified and followed before symptom onset.[9] We measured the microglia and astroglia markers CHIT1, YKL-40, and GFAP in CSF from asymptomatic mutation carriers and observed no significant differences of these markers compared with controls. The strong correlation of CHIT1 and YKL-40 in

CSF with the axonal degeneration markers NfL and pNfH further supports a close link between neuroinflammation and the degenerative phase of the diseases. Additionally, in one individual with *SOD1* mutation - only showing first EMG abnormalities and therefore representing the transition from the presymptomatic to the symptomatic phase - increased neurofilament levels but normal CHIT1 (no *CHIT1* polymorphism) and slightly increased YKL-40 (203ng/mL) were measured. This observation in this single individual could be additional evidence that axonal damage precedes neuroinflammation in ALS, but clearly awaits further confirmation. Overall, these findings suggest that neuroinflammation – as evaluated by this panel of established neuroinflammatory markers - is either not an early event in ALS and FTD or a different kind of neuroinflammation not reflected in changes in CHIT1, YKL-40 or GFAP is taking place in the initial stages of these diseases. However, our clinical findings are in agreement with studies in transgenic human mutant *SOD1* mouse models. Here also the initiation of microglia activation and astrogliosis occurs around symptom onset or even later.[19]

The observed increase of CHIT1 and YKL-40 levels in CSF of gALS patients indicates profound neuroinflammation in the symptomatic phase of ALS. Our finding in gALS is in agreement with the previously reported CHIT1 increase in CSF of sALS patients by our[20] and other groups[13,21,22]. In this context, our observation that the 24bp-duplication in exon 10 of *CHIT1,* leading to lower CHIT levels[15], did not affect axonal degeneration and disease severity in ALS patients indicates that CHIT1 itself is just a marker of the neuroinflammatory process and does not actively contribute to it. We also showed that YKL-40 is increased in both gALS and sALS which has been described only for sALS before[21–23]. In contrast, GFAP levels in CSF are not altered in gALS and sALS. Overall, our results show that the neuroinflammatory profile investigated here is similar in gALS and sALS and may indicate that neuroinflammation is a shared pathophysiological process in both gALS and sALS.

The increased levels of YKL-40 and GFAP in FTD patients also indicate profound neuroinflammation in the symptomatic phase of FTD. Our results support previous observations of increased YKL-40[17,18] and GFAP[24] in CSF of sFTD patients and we could

demonstrate similar changes in gFTD. Thus, our data indicate a similar neuroinflammatory profile in gFTD and sFTD and support the notion that it is a shared mechanism in gFTD and sFTD pathophysiology.

Although the increased CSF concentrations of the inflammatory markers studied here support profound neuroinflammation in both ALS and FTD, we observed significant differences in the neuroinflammatory profile between the diseases. Increased CSF levels of CHIT1 are characteristic of ALS whereas GFAP is increased in FTD only. YKL-40 is increased in both although higher levels were observed in sFTD but with considerable overlap. CHIT1 is thought to be a marker of microglia/macrophage activation[20] and the increased CSF levels in ALS are also in agreement with the observed peripheral monocyte and macrophage activation in ALS[25].

GFAP and YKL-40 in CSF are both considered to be markers of astrogliosis[23,26] and thus, it is surprising that they behave different in ALS and FTD. Interestingly, we observed only a very weak correlation of their CSF concentrations which is in agreement with a previous report[27]. This could indicate that different astrocyte subpopulations or a different spatial distribution are reflected by GFAP and YKL-40.

Overall, the elevated GFAP concentration in FTD indicates a higher degree or different type of astrogliosis compared with ALS. ROC analysis showed a good discriminatory power of GFAP for FTD and ALS. This is of high clinical relevance since some 15% of ALS patients suffer from concomitant FTD and their identification is essential to optimize treatment[2]. GFAP determination in CSF might be an additional tool to improve identification of FTD in ALS patients. Nevertheless, follow-up studies are needed to evaluate the diagnostic potential of GFAP to detect FTD among ALS patients.

In conclusion, our data from asymptomatic mutation carriers indicates that neuroinflammation is linked to the symptomatic phase of ALS and FTD, which is in agreement with preclinical studies in mice. We show that neuroinflammation is a shared mechanism in sporadic and genetic forms of both diseases supporting the use of mutation-based animal models to study

neuroinflammatory mechanisms. ALS and FTD are characterized by a different neuroinflammatory pattern with more severe macrophage/microglia activation in ALS and astrocytosis in FTD. These differences might be one driver for the manifestation of the ALS/FTD syndrome as FTD or ALS. GFAP in CSF is a promising biomarker candidate to identify concomitant FTD in ALS patients and may improve the diagnostic accuracy and sensitivity for treatment optimization.

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Figure legends

Fig. 1: CHIT1 is increased in gALS patients but not asymptomatic mutation carriers and gFTD. (A) CHIT1 was measured in CSF by ELISA in healthy patients without ALS mutation (controls), in asymptomatic ALS/FTD mutation carriers (Mut. carrier), in two independent cohorts (Ulm and Umeå) of genetic ALS (gALS) patients, and in genetic FTD patients (gFTD) suffering from the behavioural variant of FTD (bvFTD). Bars and whiskers are median and interquartile range, triangles are individual values. Colors indicate the status of the 24bp-duplication of *CHIT1*: no duplication (green), heterozygous (pink), homozygous (blue), no information (gray). (B) CHIT1 immunoblots from post-mortem spinal cord tissue of ALS and FTD patients carrying the *C9orf72* mutation (c9ALS and c9FTD), multiple sclerosis (MS), and non-neurodegenerative controls and (C) quantitative comparison of CHIT1 expression relative to GAPDH (mean ± SD) using Student´s *t*-test. (D) Frequency of the 24bp-dublication of *CHIT1* in the disease groups, p=0.38 (Chi-square test). (E) CHIT1 concentration in CSF in the disease groups depending on *CHIT1* genotype. (F) Comparison of CHIT1, NfL, and pNfH concentrations in CSF and age at disease onset in gALS patients (Ulm+Umeå) with or without the 24bp-duplication of *CHIT1*. Heterozygous (Het), homozygous (Homo), no duplication (WT). Bars and whiskers are median and interquartile range, triangles are individual values. Groups were compared by Kruskal-Wallis test and Dunn´s post hoc test.

Fig. 2: Increased levels of YKL-40 in ALS/FTD and GFAP in FTD but not asymptomatic ALS/FTD mutation carriers. YKL-40 and GFAP were measured by ELISA in CSF of (A, C) healthy patients without ALS mutation (controls), in asymptomatic ALS/FTD mutation carriers (Mut. carrier), in two independent cohorts (Ulm and Umeå) of genetic ALS (gALS) patients, and in genetic FTD patients (gFTD) suffering from the behavioural variant of FTD (bvFTD) and (B, D) in CSF of control patients without neurodegenerative disease (controls), sporadic

ALS, and sporadic bvFTD patients (sALS and sFTD). Bars and whiskers are median and interquartile range, triangles are individual values. Groups were compared by Kruskal-Wallis test and Dunn´s post hoc test.

Fig. 3: Receiver operating characteristic (ROC) curve analysis and estimated time course in CSF. ROC curve analysis of (A) YKL-40 and (B) GFAP concentration in CSF for control patients without neurodegenerative disease (Con, n=39 for YKL-40, n=48 for GFAP), ALS patients (sporadic and genetic, n=124 for YKL-40, n=119 for GFAP), and FTD patients (sporadic and genetic, n=36 for YKL-40, n=32 for GFAP). Cut-offs were calculated using the Youden index. AUC: area under the curve, Sens: sensitivity, Spec: specificity. (C) Disease duration at the time of lumbar puncture is shown to estimate the time course of CHIT1, YKL-40 and NfL in CSF of asymptomatic mutation carriers and genetic ALS patients. The concentrations of CHIT1, YKL-40 and NfL were normalized to the respective mean concentration of the asymptomatic mutation carriers to allow a better comparison of the magnitude of changes. The assumed time to disease onset in the asymptomatic mutation carriers was estimated using the parental age of onset. Two cases were already older than their affected relatives and for this graph we assumed that they will have their disease onset within six months. One individual with *SOD1* mutation showed early EMG abnormalities and was defined as time point 0 month.

509 **Table 1. Characteristics of genetic ALS/FTD cohort**

Characteristic	Controls (n=36)	Asymptomatic mutation carriers (n=26)	gALS Ulm (n=23)	gALS Umeå (n=43)	gFTD (n=23)	*p*-value[f]
Age (years)[a]	39.1 29.2-49.6	42.9 30.7-51.0	55.6 49.8-68.7	62.1 53.7-66.2	59.8 54.9-71.3	<0.0001
Sex (F/M)	17/19	18/8	10/13	17/26	9/14	0.15
Gene mutations	-	16x *C9orf72* 7x *SOD1* 2x *FUS* 1x *TARDBP*	11x *C9orf72* 10x *SOD1* 1x *FUS* 1x *NEK1*	22x *C9orf72* 21x *SOD1*	15x *C9orf72* 3x *MAPT* 4x *GRN*	
ALSFRS-R[a]	-	-	40 35-46[b]	n.a.	-	
FTLD-CDR[a]	-	-	-	-	9.0 5.5-16.0[b]	
Disease duration at LP (months)[a]	-	-	12.0 4.9-21.9[b]	27.8 14.1-79.5[b]	38.8 19.2-104[b]	
CSF CHIT1 (pg/mL)[a]	1433 746-2278[b]	2075 1020-3060[b]	13168 5356-38734[b]	12400 4660-23380	2520 898-6418	<0.0001
Blood CHIT1 (pg/mL)[a]	17750 11850-30950	20975 12325-30288[b]	51850 21950-60575[b,c]	21225 15150-36200[b,d]	31300 21338-53663[c]	0.0094
CSF NfL (pg/mL)[a]	202 141-283[b]	210 97-281[b]	5240 2807-12870[b]	5451 3500-8365[b]	2145 1267-4648[b]	<0.0001
CSF pNfH (pg/mL)[a]	188 188-188[b]	188 188-188[b]	2143 1362-5101[b]	2577 1423-3910	345 265-499[b]	<0.0001
CSF YKL-40 (ng/mL)[a,e]	141 126-180[b]	153 108-165[b]	220 155-409[b]	341 235-460[b]	268 220-353[b]	<0.0001
Blood YKL-40 (ng/mL)[a]	55.0 43.5-68.5	54.5 44.0-60.0[b]	62.0 41.3-136[b,c]	60.0 36.0-94.0[b,d]	67.0 47.0-101[b]	0.36
CSF GFAP (pg/mL)[a]	404 358-596[b]	414 287-517[b]	593 353-806[b]	504 316-669[b]	1350 637-1728[b]	0.0067
Blood GFAP (pg/mL)[a,e]	111 88.8-121	100 83.2-134[b]	93.8 65.8-162[b,c]	105 62.0-136[b,d]	126 82.2-231[b,c]	0.25

510 [a]values are median and interquartile range

511 [b]values are not available for all patients

512 [c]determined in blood serum

513 [d]determined in blood plasma

514 [e]age-adjusted

515 [f]Kruskal-Wallis test (Chi-square test for sex)

516 ALSFRS-R: ALS Functional Rating Scale-revised, CHIT1: chitotriosidase 1, F: female, FTLD-
517 CDR: Frontotemporal Lobar Degeneration-specific Clinical Dementia Rating, gALS: genetic
518 amyotrophic lateral sclerosis, GFAP: glial fibrillary acidic protein, gFTD: genetic
519 frontotemporal dementia, LP: lumbar puncture, M: male, n.a.: not available, NfL:
520 neurofilament light chain, pNfH: phosphorylated neurofilament heavy chain, YKL-40:
521 chitinase-3-like protein 1.

522

Table 2. Characteristics of sporadic ALS/FTD cohort

Group	Age (years)	Sex (F/M)	ALSFRS-R	FTLD-CDR	Disease duration at LP (month)	Marker
YKL-40						CSF YKL-40 (ng/mL)
Controls[a,b] (n=25)	62.0 55.2-71.1	10/15	-	-	-	129 93.4-178
sALS[a,b] (n=70)	63.0 54.8-69.0	25/45	42 36-46	-	26.6 13.8-85.8	197 125-279
sFTD[a,b] (n=26)	63.5 55.4-70.5	9/17	-	7.25 5.13-14.9[c]	42.0 18.5-60.0[c]	313 240-367
p-value[d]	0.88	0.91				<0.0001
GFAP						CSF GFAP (pg/mL)
Controls[a] (n=32)	62.8 49.5-72.4	13/19	-	-	-	792 484-1053
sALS[a] (n=64)	63.0 52.5-69.0	22/42	42 36-46	-	28.5 14.7-98.8	698 468-959
sFTD[a] (n=20)	63.5 56.0-69.8	7/13	-	6.50 4.75-14.3[c]	42.0 21.0-62.0[c]	1337 895-1968
p-value[d]	0.88	0.83				0.0001

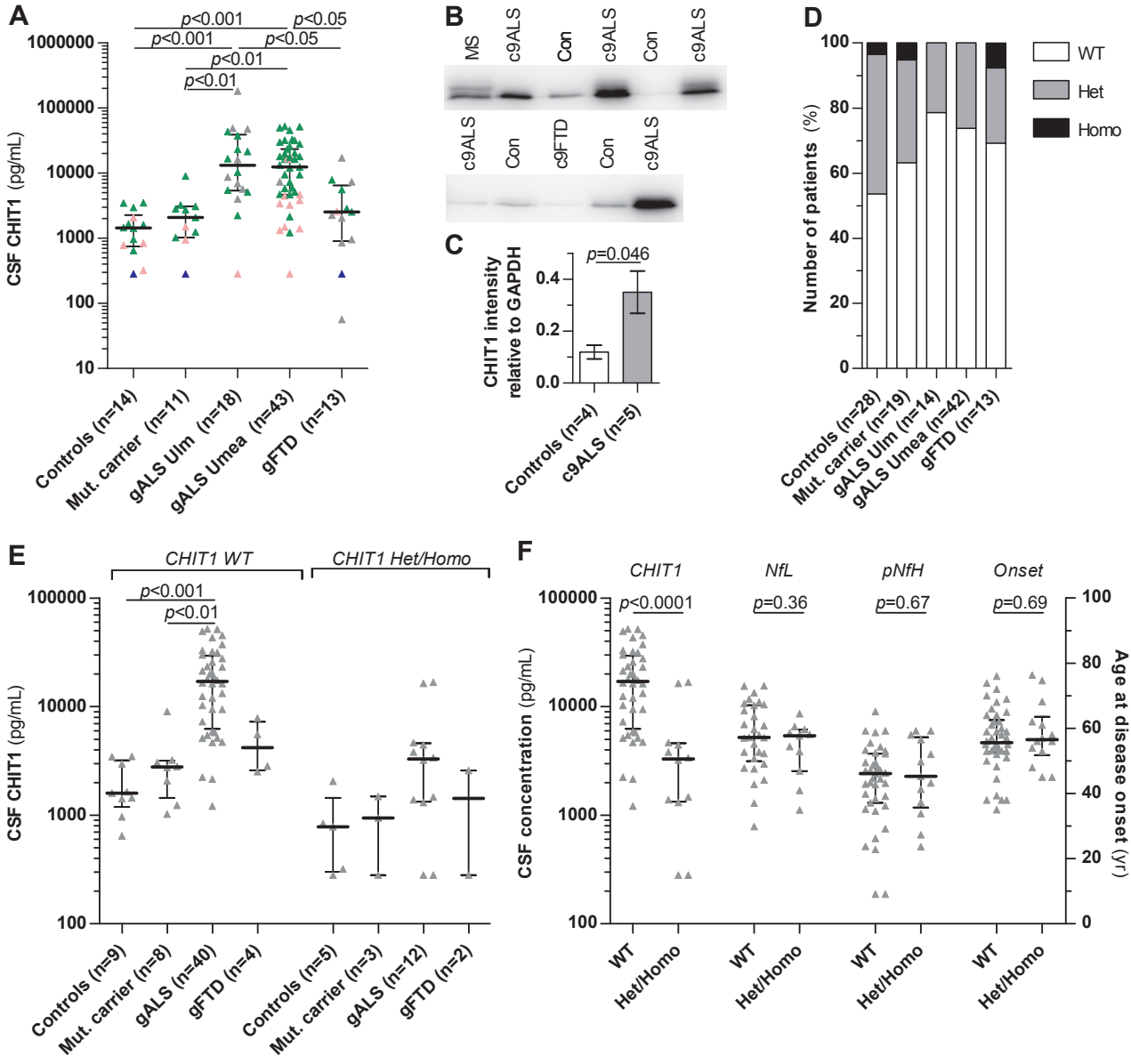
[a]values are median and interquartile range

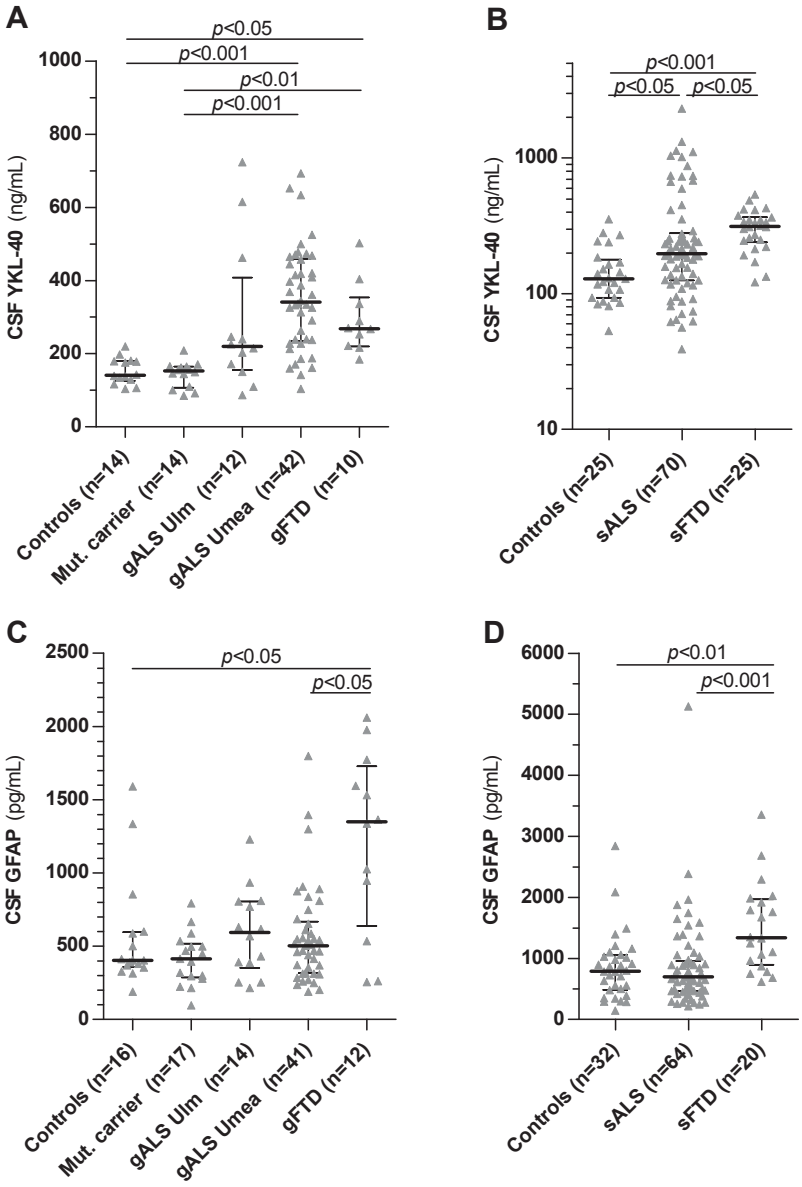
[b]age-adjusted

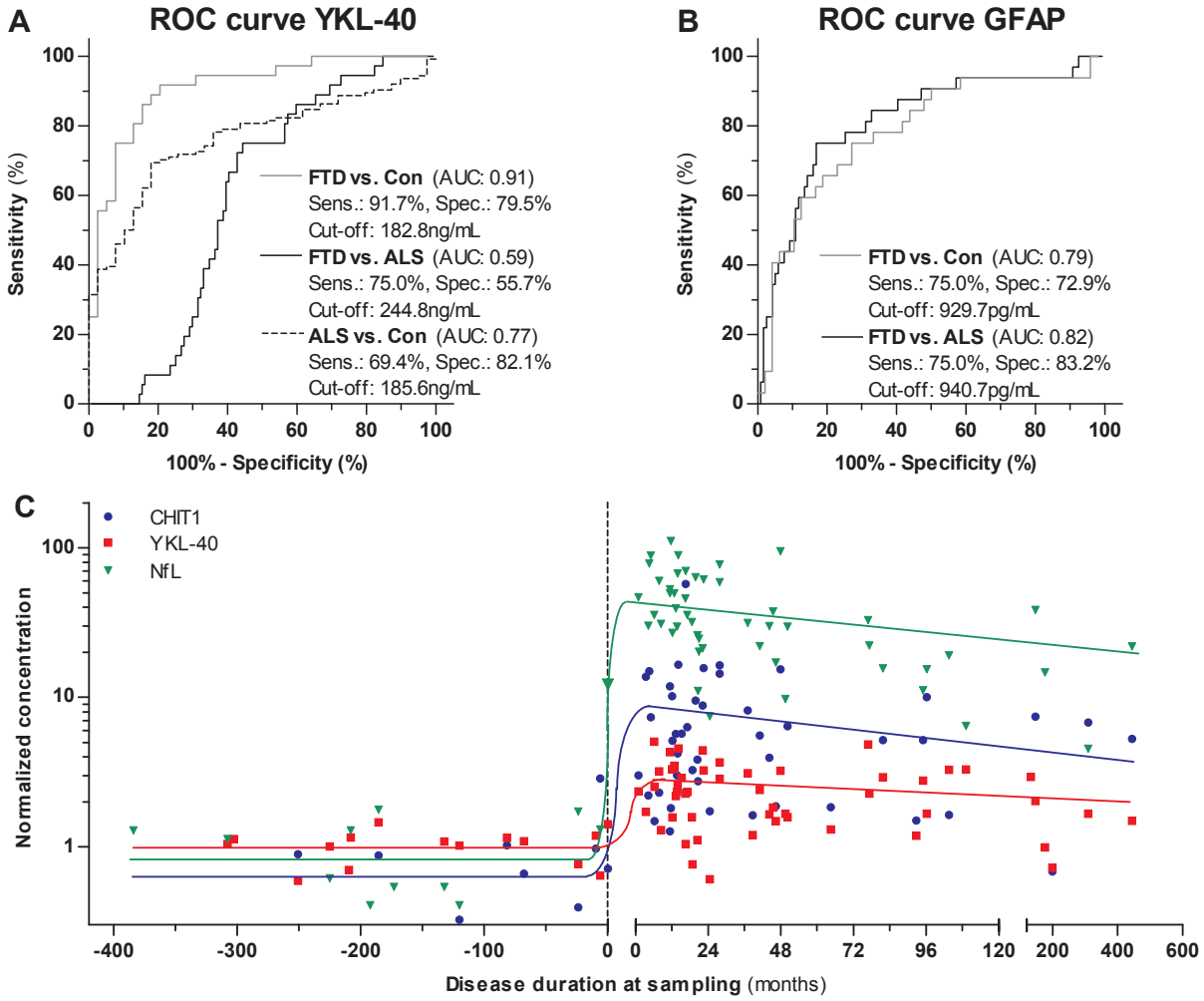
[c]values are not available for all patients

[d]Kruskal-Wallis test (Chi-square test for sex)

ALSFRS-R: ALS Functional Rating Scale-revised, F: female, FTLD-CDR: Frontotemporal Lobar Degeneration-specific Clinical Dementia Rating, GFAP: glial fibrillary acidic protein, LP: lumbar puncture, M: male, sALS: sporadic amyotrophic lateral sclerosis, sFTD: sporadic frontotemporal dementia, YKL-40: chitinase-3-like protein 1.


A
p<0.001
p<0.05
p<0.001
p<0.05
p<0.01
p<0.01
CSF CHIT1 (pg/mL)
1000000
100000
10000
1000
100
10
Controls (n=14)
Mut. carrier (n=11)
gALS Ulm (n=18)
gALS Umea (n=43)
gFTD (n=13)
B
MS
c9ALS
Con
c9ALS
Con
c9ALS
c9ALS
Con
c9FTD
Con
c9ALS
C
p=0.046
CHIT1 intensity relative to GAPDH
0.4
0.2
0.0
Controls (n=4)
c9ALS (n=5)
D
Number of patients (%)
100
80
60
40
20
0
WT
Het
Homo
Controls (n=28)
Mut. carrier (n=19)
gALS Ulm (n=14)
gALS Umea (n=42)
gFTD (n=13)
E
CHIT1 WT
CHIT1 Het/Homo
p<0.001
p<0.01
CSF CHIT1 (pg/mL)
100000
10000
1000
100
Controls (n=9)
Mut. carrier (n=8)
gALS (n=40)
gFTD (n=4)
Controls (n=5)
Mut. carrier (n=3)
gALS (n=12)
gFTD (n=2)
F
CHIT1
NfL
pNfH
Onset
p<0.0001
p=0.36
p=0.67
p=0.69
CSF concentration (pg/mL)
100000
10000
1000
100
Age at disease onset (yr)
100
80
60
40
20
0
WT
Het/Homo
WT
Het/Homo
WT
Het/Homo
WT
Het/Homo


A
p<0.05
p<0.001
p<0.01
p<0.001
CSF YKL-40 (ng/mL)
1000
800
600
400
200
0
Controls (n=14)
Mut. carrier (n=14)
gALS Ulm (n=12)
gALS Umea (n=42)
gFTD (n=10)
B
p<0.001
p<0.05
p<0.05
CSF YKL-40 (ng/mL)
1000
100
10
Controls (n=25)
sALS (n=70)
sFTD (n=25)
C
p<0.05
p<0.05
CSF GFAP (pg/mL)
2500
2000
1500
1000
500
0
Controls (n=16)
Mut. carrier (n=17)
gALS Ulm (n=14)
gALS Umea (n=41)
gFTD (n=12)
D
p<0.01
p<0.001
CSF GFAP (pg/mL)
6000
5000
4000
3000
2000
1000
0
Controls (n=32)
sALS (n=64)
sFTD (n=20)


A
ROC curve YKL-40
Sensitivity (%)
100% - Specificity (%)
FTD vs. Con (AUC: 0.91)
Sens.: 91.7%, Spec.: 79.5%
Cut-off: 182.8ng/mL
FTD vs. ALS (AUC: 0.59)
Sens.: 75.0%, Spec.: 55.7%
Cut-off: 244.8ng/mL
ALS vs. Con (AUC: 0.77)
Sens.: 69.4%, Spec.: 82.1%
Cut-off: 185.6ng/mL
B
ROC curve GFAP
Sensitivity (%)
100% - Specificity (%)
FTD vs. Con (AUC: 0.79)
Sens.: 75.0%, Spec.: 72.9%
Cut-off: 929.7pg/mL
FTD vs. ALS (AUC: 0.82)
Sens.: 75.0%, Spec.: 83.2%
Cut-off: 940.7pg/mL
C
CHIT1
YKL-40
NfL
Normalized concentration
Disease duration at sampling (months)