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Cerebrospinal fluid YKL-40 and chitotriosidase levels in frontotemporal dementia vary by clinical, genetic and pathological subtype

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Abstract

Background: Chronic glial dysfunction may contribute to the pathogenesis of frontotemporal dementia (FTD). Cerebrospinal fluid (CSF) levels of glia-derived proteins YKL-40 and chitotriosidase are increased in Alzheimer's disease (AD) but have not been explored in detail across the spectrum of FTD.

Methods: We investigated whether CSF YKL-40 and chitotriosidase levels differed between FTD patients and controls, across different clinical and genetic subtypes of FTD, and between individuals with a clinical FTD syndrome due to AD versus non-AD (FTLD) pathology (based on CSF neurodegenerative biomarkers). Eighteen healthy controls and 64 people with FTD (behavioural variant FTD, n=20; primary progressive aphasia (PPA), n=44: nfvPPA, n = 16, svPPA, n=11, lvPPA, n =14, PPA-NOS, n=3) were included. 10/64 had familial FTD, with mutations in *GRN* (n=3), *MAPT* (n=4), or *C9orf72* (n=3). 15/64 had neurodegenerative biomarkers consistent with AD pathology. Levels were measured by immunoassay and compared using multiple linear regressions. We also examined relationships of YKL-40 and chitotriosidase with CSF total tau (T-tau), phosphorylated tau 181 (P-tau) and β-amyloid 1-42 (Aβ42), with each other, and with age and disease duration.

Results: CSF YKL-40 and chitotriosidase levels were higher in FTD, particularly lvPPA (both) and nfvPPA (YKL-40), compared with controls. *GRN* mutation carriers had higher levels of both proteins than controls and *C9orf72* expansion carriers, and YKL-40 was higher in *MAPT* mutation carriers than controls. Individuals with underlying AD pathology had higher YKL-40 and chitotriosidase levels than both controls and those with likely FTLD pathology. CSF YKL-40 and chitotriosidase levels were variably associated with levels of T-tau, P-tau and Aβ42, and with each other, depending on clinical syndrome and underlying pathology. CSF YKL-40 but not chitotriosidase was associated with age, but not disease duration.

Conclusion: CSF YKL-40 and chitotriosidase levels are increased in individuals with clinical FTD syndromes, particularly due to AD pathology. In a preliminary analysis of genetic groups, levels of both proteins are found to be highly elevated in FTD due to *GRN* mutations, while YKL-40 is increased in individuals with *MAPT* mutations. As glia-derived protein levels generally correlate with T-tau and P-tau levels, they may reflect the glial response to neurodegeneration in FTLD.

Keywords

Astrocytes, Biomarkers, Cerebrospinal fluid, CHI3L1, chitotriosidase, Frontotemporal dementia, Microglia, Neuroinflammation, Progranulin, YKL-40

Background

Frontotemporal dementia (FTD) causes progressive changes in behaviour (behavioural variant FTD, bvFTD), or language (primary progressive aphasia, PPA) and some individuals have concurrent motor neuron disease (MND) or an atypical parkinsonian disorder such as progressive supranuclear palsy (PSP) or corticobasal syndrome (CBS) [1]. Pathologically, most individuals have frontotemporal lobar degeneration (FTLD) with tau inclusions (FTLD-tau) or transactive response DNA binding protein-43 (TDP-43) inclusions (FTLD-TDP), although some, particularly those with logopenic variant PPA (lvPPA), have underlying Alzheimer's disease (AD) pathology [2,3]. Around two thirds of cases are sporadic, but one third are familial, associated most commonly with mutations in progranulin (*GRN*), microtubule associated protein tau (*MAPT*) or chromosome 9 open reading frame 72 (*C9orf72*) [1]. Biomarkers are currently lacking that reliably differentiate the pathological changes *in vivo* in sporadic FTD, can predict onset of disease and guide timely initiation of future treatments in familial FTD, or assess treatment response in future clinical trials.

There is growing evidence that chronic neuroinflammation plays a role in FTD, especially in familial FTD secondary to mutations in *GRN* [4–7], but also in people with *MAPT* [8–10] and *C9orf72* [11–16] mutations, and in sporadic FTD [6,17–20]. Histological studies of brain tissue from patients with FTD implicate excessive microglial activation [9,10,21–29] and astrocytosis [26,28] but also microglial dystrophy [21] in disease pathogenesis. Although microglia and astrocytes may initially be helpful in neurodegenerative diseases through phagocytosis of aggregated proteins and dying neurons and remodeling of synapses, over time they may become harmful, through chronic activation and release of pro-inflammatory cytokines and other toxic proteins. Accelerated microglial senescence, dysfunction and reduced phagocytic and supportive capacity may also exacerbate neuronal demise [30]. Inflammatory markers associated with these processes, particularly proteins derived from glial cells, may be detectable and altered in blood or CSF, and could be useful biomarkers of chronic neuroinflammation and disease pathogenesis in FTD.

Although it is now well-established that levels of several glia-derived proteins are raised in CSF during various stages of neurodegenerative diseases such as AD or MND, fewer studies have explored how levels are altered in FTD. Three glia-derived proteins have been most extensively explored in neurodegenerative diseases: soluble triggering receptor expressed on myeloid cells 2 (sTREM2), YKL-40 (also known as chitinase-3-like protein 1, CHI3L1), and chitotriosidase (also known as CHIT1). TREM2 is an innate immune receptor expressed by myeloid cells, including microglia and peripheral macrophages [31], and is involved in phagocytosis, survival and migration of microglia. A soluble fragment (sTREM2) is cleaved and detectable in CSF and blood [32]. YKL-40 is a pro-inflammatory molecule released predominantly by activated astrocytes (and to a lesser extent by microglia) into the CSF and by activated peripheral macrophages into blood, which stimulates production of cytokines, and regulates macrophage, microglial and astrocytic function, endothelial cell migration and tumour angiogenesis [33]. Chitotriosidase is a chitin-degrading enzyme expressed by activated microglia (but not by astrocytes) in CSF [34], and by peripheral macrophages in blood [35]. It induces activation of a pro-inflammatory microglial phenotype [34] and has a range of other immunomodulatory functions, including stimulation of chemotactic factors, fibrosis and tissue remodeling [36].

We have recently shown that although CSF sTREM2 levels are not raised in a mixed cohort of individuals with a diagnosis of FTD compared with controls, they are higher in certain subgroups of FTD, such as individuals with a clinical syndrome consistent with FTD but underlying AD pathology, and in symptomatic *GRN* mutation carriers [37]. This has implications for the use of glia-derived proteins as CSF biomarkers in clinical trials for a condition as diverse as FTD. CSF YKL-40 and chitotriosidase levels have not been compared across all the clinical and the main genetic subtypes of FTD or correlated within FTD subgroups with levels of validated CSF neurodegenerative biomarkers used in clinical practice: total tau (T-tau), phosphorylated tau-181 (P-tau) and amyloid beta 1-42 (Aβ42).

This study therefore set out to examine how CSF YKL-40 and chitotriosidase levels differ between individuals with a clinical diagnosis of FTD and cognitively normal controls, and between different clinical and genetic subtypes of FTD. We also aimed to clarify how CSF YKL-40 and chitotriosidase levels differ between individuals with similar clinical FTD syndromes but different underlying pathologies: FTLD versus AD, based on the CSF tau/Aβ42 biomarker profile. We also aimed to establish whether YKL-40 or chitotriosidase levels are associated with levels of T-tau, P-tau or Aβ42 in CSF, and to ascertain the relationship between YKL-40 and chitotriosidase levels, across the spectrum of FTD. Finally, we aimed to assess how YKL-40 and chitotriosidase levels are associated with parameters that may affect glial function and are relevant for future clinical trials, such as age, disease duration and sex.

Methods

Participants

The cohort consisted of 64 consecutively recruited individuals with dementia meeting consensus diagnostic criteria for either bvFTD [38] or PPA [39], and 18 healthy cognitively normal controls (as per [37], with the addition of an extra control, recruited subsequent to that study). Cases with additional motor neurone disease were not included in the study. The study was approved by the local NHS Research Ethics Committee and the Health Research Authority. All individuals gave informed written consent.

Within the patient group, 20 had bvFTD, 16 nonfluent variant PPA (nfvPPA), 11 semantic variant PPA (svPPA), 14 logopenic variant PPA (lvPPA) and three had a PPA syndrome not otherwise specified (PPA-NOS; not fulfilling criteria of any of the other PPA phenotypes). All participants with FTD were genetically screened for all known FTD causative mutations, including the *C9orf72* expansion. Ten individuals were found to have familial FTD, with mutations in *GRN* (n=3: two C31fs, one S78fs), *MAPT* (n=4: two 10+16, two R406W) or *C9orf72* (n=3). All familial cases had a diagnosis of bvFTD except two

individuals with *GRN* mutations who had nfvPPA. Demographics of the cohort are displayed in Table 1. Disease duration was calculated as the time, in years, between age at clinical onset of symptoms and date of CSF collection.

There was no difference in age at CSF collection between the FTD and control groups (P = 0.854), but the svPPA subgroup were younger than the nfvPPA (P = 0.018) and lvPPA (P = 0.031) subgroups. There was no significant difference in age between any of the genetic subgroups or when compared with controls. There was a higher proportion of males in the FTD group than in the control group (P = 0.014), and a higher proportion of males in the bvFTD subgroup versus all other clinical subgroups and controls (P = 0.006), other than the PPA-NOS group, where all 3 participants were male (Table 1). There was no significant difference in disease duration between any of the clinical subgroups (P = 0.105).

CSF collection, processing and biomarker analysis

For all participants, CSF was collected and stored using standardised procedures [37,40]. Samples were collected by lumbar puncture in polypropylene tubes, which were immediately transferred to the laboratory. Samples were then centrifuged and the supernatant aliquoted and stored at − 80 °C within 30 minutes of arrival. Levels of T-tau, P-tau and Aβ42 were measured in CSF using commercially available INNOTEST sandwich enzyme-linked immunosorbent assays (Fujirebio Europe, Gent, Belgium).

CSF YKL-40 levels were measured using the commercially available Human YKL-40 Immunoassay Kit on the Mesoscale Discovery (MSD, Rockville, MD, USA) platform, with all samples assayed in duplicate and measured on the same day by a single operator using the same reagents. Briefly, CSF samples were diluted 1 in 400 with dilution buffer, and the provided standard reconstituted 1 in 20 using dilution buffer and serially diluted 1 in 4 to produce concentrations ranging from 50,000 to 12.2 pg/mL. 150 μL blocking agent was added to each well, and plates sealed and incubated at room temperate shaking at

500 rpm for 1 hour. Plates were washed 3 times with 300 μL per well of PBS-T, then 50 μL of either diluted CSF sample, standard or blank (dilution buffer) was added to each well (pre-coated with capture antibody), and plates were sealed and incubated at room temperate shaking at 500 rpm for 2 hours. Plates were washed 3 times with 300 μL of PBS-T and 25 μL of detection antibody solution (diluted to 1 in 50) added per well, then sealed and incubated at room temperate shaking at 500 rpm for 2 hours. After a further 3 washes with PBS-T, 150 μL of Read Buffer T (diluted 1 in 2) was added to each well and the plate immediately analysed on the SECTOR Imager using a 4-parameter logistic model with averaged replicates.

CSF chitotriosidase levels were measured using the commercially available CircuLex Human ELISA Kit (MBL International, MA, USA) with all samples assayed in duplicate and measured on the same day by a single operator using the same reagents. Briefly, CSF samples were diluted 1 in 5 with dilution buffer and the provided standard was diluted to produce concentrations ranging from 3600 to 56.25 pg/mL. 100 μL of either diluted CSF sample, standard or blank (dilution buffer) was added to each well, and plates sealed and incubated at room temperature for 1 hour shaking at 300 rpm, then washed 4 times with 350 μL wash buffer. 100 μL of HRP conjugated detection antibody was added and plates sealed and incubated at room temperature for 1 hour shaking at 300 rpm, then washed 4 times with 350 μL wash buffer. 100 μL of substrate agent was added to each well and plates were sealed, covered in foil and incubated for 15 minutes at room temperature shaking at 300 rpm. Finally, 100 μL of stop solution was added to each well in the same order as the substrate agent, and plate absorbance read immediately on a microplate reader at dual wavelengths of 450/540 nm. The concentration of chitotriosidase in each sample was calculated using a four-parameter fitting method based on the standard curve, using values which were blank corrected and averaged over replicates.

Participant stratification

We performed three separate group comparisons:

1. **By clinical syndrome**, comparing bvFTD, nfvPPA, svPPA, lvPPA, PPA-NOS, and controls.

2. **By genetic group,** comparing those with *GRN* mutations, *MAPT* mutations, *C9orf72* expansions, and controls

3. **By pathological group,** comparing those with likely Alzheimer's disease, those with likely FTLD pathology, and controls. We used levels of CSF T-tau and Aβ42 to calculate the T-tau/Aβ42 ratio for each participant to perform this stratification, classifying all individuals with dementia based on the CSF T-tau/Aβ42 ratio, with a cut-off of ≥1.0 (AD biomarker-positive, indicating likely AD) and <1.0 (AD biomarker-negative, indicating likely FTLD) [37,40]; Table 2. The cognitively normal controls formed a comparison group with all having a CSF T-tau/Aβ42 ratio of <1.0. No significant difference in age at CSF was seen between these three groups ($P > 0.050$), but disease duration at CSF was lower in the AD biomarker-positive subgroup than the AD biomarker-negative subgroup ($P = 0.037$). There were significantly more males in the AD biomarker-negative subgroup (73.4%) than in controls (38.9%) and the AD biomarker-positive subgroup (60.0%) ($P = 0.032$).

Statistical analysis

For YKL-40, levels were detectable in CSF of all individuals, so analyses were performed on all 18 controls and 64 individuals with FTD. For chitotriosidase, three individuals (one control, one sporadic bvFTD and one sporadic nfvPPA) had persistently undetectable levels of chitotriosidase in CSF, despite assaying their samples again at 1 in 5 dilution and using neat CSF. Approximately 6% of the population possess a homozygous 24-bp duplication in exon 10 of the CHIT1 gene which leads to a complete enzymatic deficiency of chitotriosidase [41]. These three individuals were very likely to be carriers of this mutation and their levels would bias comparisons of the groups if included (or assigned the lower limit of detection), hence they were excluded from the chitotriosidase analysis, leaving 17 controls and 62 individuals with dementia.

All analyses were carried out using STATA14 (Stata Corporation, College Station, TX), with a significance threshold of $P<0.05$. Shapiro Wilk tests of raw values and Q-Q plots of residuals from multivariable linear regressions were used to test assumptions of normality.

Assessment of residuals in multivariable linear regression analyses of YKL-40 across groups revealed these were normally distributed and so met the assumptions required for parametric multivariable linear regression analysis. However, the same assessment for chitotriosidase revealed that residuals were not normally distributed, and so chitotriosidase values were natural log (Ln) transformed, which then met assumptions required for multivariable linear regression analysis. Multivariable linear regressions were used to compare YKL-40 and Ln(chitotriosidase) levels between groups (FTD versus controls and between clinical, pathological and genetic subgroups and versus controls), adjusting for age and sex in all analyses, and for disease duration in analyses involving comparison of disease groups (but not for genetic subgroups due to small sample size). Post hoc pairwise tests were used to compare individual subgroups.

In each group (except genetic subgroups due to small sample size), multivariable linear regressions were used to investigate the association between:
a) YKL-40 or Ln(chitotriosidase) levels and levels of CSF T-tau, P-tau and Aβ42, adjusted for age and sex (for the control group) and for age, sex and disease duration (for disease groups). Due to a non-linear relationship between T-tau levels and YKL-40 and chitotriosidase levels, T-tau values were Ln transformed and all regression analyses performed using Ln(T-tau).
b) Ln(chitotriosidase) and Ln transformed YKL-40 levels (due a to non-linear relationship between raw values), adjusted for age and sex, and for disease groups, disease duration as well.
c) YKL-40 or Ln(chitotriosidase) and both age at CSF collection (adjusted for sex in the control group and both sex and disease duration in disease groups) and disease duration at CSF collection (for disease groups, adjusted for age and sex).

Mann-Whitney U tests were used to compare protein levels between males and females in each group (but not within genetic subgroups due to small sample size).

Results

CSF YKL-40 and chitotriosidase levels are higher in certain FTD syndromes than controls

CSF YKL-40 levels were higher overall in individuals with a clinical FTD syndrome than in controls (mean (SD) = 134 (53) versus 108 (30) ng/ml, P = 0.019; Fig. 1a, Tables 1 and 3). However, this varied by clinical subtype (Fig. 1b; Tables 1 and 3): YKL-40 levels were highest in individuals with nfvPPA (149 (57) ng/ml, P = 0.021 versus controls) and lvPPA (147 (64) ng/ml, P = 0.036). Although individuals with PPA-NOS had similarly high YKL-40 levels (146 (45) ng/ml), the small size of this group meant that the difference from controls was not statistically significant (P = 0.124). No significant differences were seen between YKL-40 levels in bvFTD or svPPA subgroups compared with controls, and there were no significant differences between clinical subgroups (Fig. 1b).

CSF chitotriosidase levels were also significantly higher overall in individuals with a clinical FTD syndrome compared with controls (Fig. 2a, Tables 1 and 3; FTD: mean (SD) = 3795 (4358) versus controls: 1762 (1098) pg/ml, P = 0.038). Although a trend towards higher levels was seen in all clinical subgroups (Fig. 2b; Tables 1 and 3), this only reached statistical significance in lvPPA (5240 (5039) pg/ml, P = 0.017) and there were no significant differences between clinical subgroups.

CSF YKL-40 and chitotriosidase levels differ by underlying gene mutation in FTD

GRN mutation carriers had significantly higher levels of YKL-40 compared with controls (mean (SD) = 226 (42) versus 108 (30) ng/ml, P <0.001; Fig. 1c) and compared with *C9orf72* expansion carriers (99 (40) ng/ml, P = 0.001), with a trend to a higher level compared with *MAPT* mutation carriers (150 (69) ng/ml,

P = 0.066). *MAPT* mutation carriers also had significantly higher YKL-40 levels than controls (*P* = 0.046; Fig. 1c), with a trend to higher levels compared with *C9orf72* expansion carriers (*P* = 0.055).

GRN mutation carriers had much higher levels of chitotriosidase compared with controls (mean (SD) = 9492 (5143) versus 1762 (1098) pg/ml, *P* <0.001), *MAPT* mutation carriers (2770 (1664) pg/ml, *P* = 0.034) and *C9orf72* expansion carriers (1688 (1345) pg/ml, *P* = 0.002; Fig. 2c). However, in contrast to YKL-40, *MAPT* mutation carriers had more similar chitotriosidase levels to controls (*P* = 0.104) and *C9orf72* expansion carriers (*P* = 0.136).

CSF YKL-40 and chitotriosidase levels are higher in FTD syndromes due to underlying AD pathology

Levels of both YKL-40 and chitotriosidase were highest in the AD biomarker-positive subgroup (YKL-40 mean (SD) = 163 (67) ng/ml; chitotriosidase = 5975 (4616) pg/ml) with significantly higher levels of both proteins in this subgroup compared with the AD biomarker-negative subgroup (YKL-40: 125 (45) ng/ml; *P* = 0.048, Fig. 1d; chitotriosidase: 3336 (4121) pg/ml, *P* = 0.007, Fig. 2d; Tables 2 and 3) and also compared with controls (YKL-40: 108 (30) ng/ml, *P* = 0.001, Fig. 1d; chitotriosidase: 1762 (1098) pg/ml, *P*<0.001, Fig. 2d; Tables 2 and 3). There was a non-significant trend to a higher level of each protein in the AD biomarker-negative subgroup versus controls (YKL-40: *P* = 0.091, Fig. 1d; chitotriosidase: *P* = 0.194, Fig. 2d; Tables 2 and 3).

CSF YKL-40 and chitotriosidase are variably associated with T-tau, P-tau and Aβ42

Associations between levels of CSF neurodegenerative biomarkers T-tau, P-tau and Aβ42 and levels of CSF YKL-40 (Fig. 3) or chitotriosidase (Fig. 4) varied according to clinical diagnosis and CSF biomarker profile (i.e. underlying pathology). In controls, CSF YKL-40 and chitotriosidase were not significantly associated with any biomarker. In the overall FTD (dementia) group, CSF YKL-40 and chitotriosidase levels were significantly positively associated with both T-tau and P-tau levels, and there was a small, negative association of chitotriosidase with Aβ42 levels. For YKL-40: T-tau β (95% CI) = 42.996 (24.878,

61.113), *P*<0.001 (Fig. 3a); P-tau β = 0.722 (0.339, 1.105, *P*<0.001 (Fig. 3b); Aβ42 β = -0.003 (-0.051, 0.045), *P* = 0.907 (Fig. 3c). For chitotriosidase: T-tau β = 0.668 (0.306, 1.030), *P*<0.001 (Fig. 4a); P-tau β = 0.009 (0.001, 0.016), *P* = 0.028 (Fig. 4b); Aβ42 β = -0.0009 (-0.0017, -0.0002), *P* = 0.044 (Fig. 4c).

Most clinical subgroups showed positive slopes for the association between YKL-40 and T-tau or P-tau levels (Fig. 3d and 3e), but this only reached significance in certain subgroups. YKL-40 levels were significantly positively associated with T-tau levels in bvFTD (β (95% CI) = 109.3 (80.6, 138.1) *P*<0.001) and nfvPPA (β = 69.9 (21.2, 118.6), *P*=0.009), and with P-tau levels in bvFTD (β = 1.746 (0.607, 2.885), *P* = 0.005) and lvPPA (β = 1.020 (0.199, 1.841), *P* = 0.020). YKL-40 levels were not associated with Aβ42 levels in most subgroups, except in lvPPA (Fig. 3f), where there was a significant positive association (β = 0.274 (0.043, 0.506), *P* = 0.025). Chitotriosidase levels were positively associated with T-tau or P-tau levels only in certain clinical subgroups (Fig. 4d and 4e). There was a significant association between chitotriosidase and T-tau levels in bvFTD (β = 1.312 (0.132, 2.491) *P* = 0.003) and lvPPA (β = 0.937 (0.231, 1.642), *P* = 0.015) and with P-tau levels in lvPPA (β = 0.014 (0.001, 0.027), *P* = 0.043). Although most clinical subgroups except lvPPA had borderline negative associations between chitotriosidase and Aβ42 levels, these did not reach significance (Fig. 4f).

Although both pathological subgroups (AD biomarker-positive and AD biomarker-negative) and controls seemed to have positive associations between YKL-40 and T-tau and P-tau levels (Fig. 3g and 3h), associations were only significant in the AD biomarker-negative subgroup: T-tau β (95% CI) = 62.064 (37.676, 86.451), *P*<0.001; P-tau β = 1.055 (0.261, 1.849) *P* = 0.010). The AD biomarker-positive subgroup had a significant, positive association between YKL-40 and Aβ42 levels (β = 0.226 (0.011, 0.442), *P* = 0.041, Fig. 3i). There were no significant associations between chitotriosidase levels and T-tau, P-tau or Aβ42 levels in any pathological subgroup or controls (Fig. 4g-i), although there was a trend towards a positive association between chitotriosidase and T-tau in the AD biomarker-negative subgroup (β = 0.631 (-0.003, 1.265); *P* = 0.051, Fig. 4g).

CSF chitotriosidase is positively associated with YKL-40 in FTD

CSF chitotriosidase levels were positively associated with YKL-40 levels within the whole cohort (β (95% CI) = 1.008 (0.507, 1.509), P<0.001) and in the overall FTD (dementia) group (β = 1.094 (0.508, 1.680), P<0.001, Fig. 5a) but not in controls (β = -0.203 (-1.605, 1.199), P = 0.774, Fig. 5a). Levels of both proteins were also positively associated in most clinical subgroups (Fig. 5b) but reached significance only in bvFTD β = 1.369 (0.399, 2.340), P = 0.007) and nfvPPA (β = 1.388 (0.034, 2.742) P = 0.045). Levels were positively associated in the AD biomarker-negative subgroup (β = 1.226 (0.556, 1.896), P = 0.001; Fig. 5c) but this did not reach significance in the AD biomarker-positive subgroup (β = 0.275 (-0.781, 1.332), P = 0.604; Fig. 5c).

CSF YKL-40 but not chitotriosidase levels are associated with age

CSF YKL-40 levels were positively associated with age at CSF in the whole cohort (β (95% CI) = 1.989 (0.352, 3.625), P = 0.018). A similar magnitude of association was seen in the FTD group (β = 1.864 (0.059, 3.670), P = 0.043, Fig. 6a) and the control group (β = 2.467 (-1.074, 6.007), P = 0.169; Fig. 6a). Although most of the clinical subgroups (apart from nfvPPA and PPA-NOS; Fig. 6b) and both pathological subgroups appeared to have a positive slope for the association between YKL-40 levels and age, none reached significance. Chitotriosidase levels were not significantly associated with age in either the whole cohort (β = 0.007 (-0.024, 0.378), P = 0.661) or the FTD (β = 0.004 (-0.064, 0.073), P = 0.904; Fig. 6c) or control (β = 0.008 (-0.026, 0.042) P = 0.628; Fig. 6c) groups, or in any of the clinical (Fig. 6d) or pathological subgroups.

There were no significant associations between either YKL-40 (Fig. 7a) or chitotriosidase (Fig. 7c) levels and disease duration in the FTD group (YKL-40: β = -1.874 (-5.403, 1.655), P = 0.292; chitotriosidase: β = -0.016 (-0.082, 0.051) P = 0.631) or within any of the clinical (Fig. 7b and 7d) or pathological subgroups.

CSF YKL-40 and chitotriosidase levels did not differ significantly between males and females in the whole cohort or in FTD or control groups, or in any of the clinical or pathological subgroups.

Discussion

This study shows that levels of two glia-derived proteins, YKL-40 and chitotriosidase, are raised in the CSF of individuals with a clinical diagnosis of FTD compared with controls. However, levels are not consistently raised across all clinical subtypes, with highest YKL-40 levels in lvPPA and nfvPPA, and highest chitotriosidase levels in lvPPA. Individuals with a clinical syndrome consistent with FTD but a CSF neurodegenerative biomarker profile consistent with AD pathologically have particularly high levels of both proteins compared with controls, and higher levels than individuals with a diagnosis of FTD and non-AD like CSF biomarkers (likely FTLD), who have a non-significant trend to higher levels than controls. In a smaller subgroup analysis, both YKL-40 and chitotriosidase levels are highly elevated in FTD due to *GRN* mutations and YKL-40 levels are also elevated in FTD due to *MAPT* mutations. Associations between YKL-40 and chitotriosidase levels, and with T-tau, P-tau and Aβ42 levels, vary depending on clinical diagnosis and CSF biomarker profile. CSF YKL-40 levels, but not chitotriosidase levels, are associated with age, and neither are associated with disease duration.

Raised CSF YKL-40 levels have previously been demonstrated in several FTD cohorts versus controls [33,42–49], including in familial FTD [48], and compared with individuals with primary psychiatric diagnoses [50]. In contrast, few studies have examined CSF chitotriosidase levels in FTD. One study found higher chitotriosidase levels in FTD (in cases without CSF biomarker or pathological confirmation) compared with healthy controls [51], but another found similar levels to controls in a mixed familial FTD cohort [48]. Our results and results from previous biomarker and histological studies suggest significant glial activation is present in individuals with clinical diagnoses of FTD. However, previous biomarker studies have included individuals with different clinical syndromes, gene mutations and underlying pathologies (or co-pathologies) within FTD cohorts. This has limited

our understanding of how glia-derived proteins vary in CSF across the spectrum of FTD, and how these biomarkers may be useful for clinical trials targeting different FTD subgroups. Our study therefore aimed to elucidate how levels of YKL-40 and chitotriosidase vary across the spectrum of FTD by examining these proteins at the subgroup level.

CSF YKL-40 and chitotriosidase levels were significantly raised in several, but not all, clinical subtypes of FTD and there was significant variability in levels within clinical subgroups. The highest YKL-40 levels were seen in lvPPA and nfvPPA, but the PPA-NOS subgroup also had high levels (likely not reaching significance when compared with controls due to small sample size). The highest chitotriosidase levels were in lvPPA, but other clinical subgroups showed non-significant trends towards higher levels than controls. There were no significant differences in levels between clinical subgroups. Very few studies have explored this previously: one study examining CSF YKL-40 levels in bvFTD, nfvPPA, svPPA, CBS and PSP compared with controls found raised levels in all syndromes (except PSP) and no significant differences between FTD subgroups, although individuals with lvPPA were not delineated from an accompanying typical AD group [43]. A study of CSF chitotriosidase levels in FTD found no significant difference between bvFTD and PPA, or between PPA subtypes, although PPA subgroups were much smaller than in our cohort and lacked biomarker or pathological correlation [51]. Different clinical subtypes of FTD may have widely differing pathologies, co-pathologies and disease mechanisms and hence differing degrees of glial activation which could affect YKL-40 and chitotriosidase release. In particular, patients with MND have much higher CSF YKL-40 levels [46,48,52–55] and CSF chitotriosidase levels or activity [34,48,51,54–57] than controls, and higher chitotriosidase levels than in FTD [51], so our study did not include individuals with FTD-MND to avoid confounding results. The majority (78%) of our lvPPA subgroup had a CSF biomarker profile consistent with AD (rather than FTLD), and our nfvPPA subgroup contained two individuals with AD-like CSF biomarkers and two individuals with *GRN* mutations. In contrast, most individuals with bvFTD or svPPA had non AD-like biomarkers and either a smaller percentage of (bvFTD) or no *GRN*

mutations. We therefore hypothesise that the particularly high YKL-40 and chitotriosidase levels in lvPPA and high YKL-40 levels in nfvPPA may be due to more pronounced glial activation in individuals with underlying AD pathology and *GRN* mutations.

We were able to explore this further by stratifying our FTD cohort by CSF neurodegenerative biomarker profile (T-tau/Aβ42 ratio) rather than by clinical diagnosis. This was helpful in a previous study to demonstrate that CSF sTREM2 levels are raised in individuals with a clinical diagnosis of an FTD syndrome but AD-like CSF biomarkers, particularly lvPPA, compared with controls, but not in those with likely FTLD [37]. By repeating this approach, we confirm that there are also much higher levels of YKL-40 and chitotriosidase in the CSF of individuals with an FTD syndrome but AD-like CSF biomarkers compared with controls, and higher levels than in individuals with non AD-like CSF (i.e. likely FTLD). Patients with typical amnestic AD have elevated levels of glia-derived proteins in CSF compared with controls, including YKL-40 [42,47,66–69,58–65], sTREM2 [70–73], chitotriosidase [51,59,74,75], glial fibrillary acidic protein [76–78] and S100beta [79–81]. This suggests there is pronounced astrocytic and microglial activation in association with AD pathology. The soluble phosphorylated tau species found in patients with amnestic AD are highly toxic to microglia, resulting in pronounced microglial dysfunction and dystrophy [82] and tau oligomers co-localise with microglia, astrocytes and pro-inflammatory cytokines in patients with AD and FTLD [26]. Patients with clinical FTD but underlying AD pathology would therefore also be expected to have very high levels of glia-derived proteins compared with controls, and data from this study and our previous study [37] support this.

Although CSF YKL-40 and chitotriosidase levels did not differ significantly between individuals with FTD and non AD-like CSF biomarkers (likely FTLD) and controls, there was a trend towards higher levels of both proteins in this group. There was also significant intra-group variability in protein levels, particularly for chitotriosidase, suggesting glial activation may vary considerably according to the

FTLD subtype or disease mechanism. Other studies have explored this by stratifying pathologically confirmed FTLD groups and found higher CSF YKL-40 levels in both FTLD-tau and FTLD-TDP than in individuals with subjective memory impairment, and in FTLD-tau compared with AD [43,44,49]. One study found higher YKL-40 levels in 'pure' FTLD-tau (excluding AD pathology) than in patients with FTLD-TDP or AD [44], although others have found higher levels in FTLD-TDP (but not FTLD-tau) compared with controls [43,45]. Differences in the number of genetic FTD cases in FTLD subgroups or inclusion of individuals with co-pathology are likely to have contributed to these disparities between studies. FTLD-TDP cohorts have included patients with concurrent MND [45], or patients with *GRN* mutations [43] who we demonstrate have very high levels of both YKL-40 and chitotriosidase. FTLD-tau cohorts have included differing numbers of *MAPT* cases, who we show have particularly raised YKL-40 levels. This variability in levels according to pathology or disease mechanism has implications for the use of inflammatory proteins as fluid biomarkers in both research studies and clinical trials for a disease as pathologically diverse as FTD. It also emphasizes the importance of detailed stratification of cohorts or use of CSF biomarker or pathological correlation in biomarker studies of FTD.

Although we were unable to divide our cohort into FTLD subtypes due to a lack of cases with pathological confirmation, we were able to include a small number of individuals with familial FTD, enabling an exploratory analysis of CSF YKL-40 and chitotriosidase levels in a small number of individuals with known pathology (FTLD-TDP: *GRN* or *C9orf72* mutations or FTLD-tau: *MAPT* mutations), and also differing disease mechanisms despite similar pathology (*GRN* and *C9orf72* mutations). *GRN* mutation carriers had very elevated YKL-40 and chitotriosidase levels and *MAPT* mutation carriers had high YKL-40 levels compared with controls. Very few studies have examined glia-derived CSF biomarkers in individuals with genetic FTD. In a recent study, CSF YKL-40 levels, but not CSF chitotriosidase levels, were significantly elevated in 23 familial FTD cases (combining *C9orf72*, *GRN* or *MAPT* mutation carriers) compared with controls [48]. However, genetic subgroup analyses were not performed to analyse differences between mutation types and the genetic group contained a

significant proportion of patients with *C9orf72* expansions (15/23) (who we found to have similar levels of both proteins to controls), which may have influenced results. The highly elevated levels of YKL-40 and chitotriosidase in our *GRN* mutation group is consistent with multiple studies showing elevated levels of other inflammatory markers in *GRN* mutation carriers [6,19,37,83–85]. *GRN* haploinsufficiency results in significant microglial dysfunction and activation [4,21,86], which could lead to excessive YKL-40 and chitotriosidase release as a mutation-specific effect, exacerbated by the general glial response to neurodegeneration, perhaps explaining the higher levels in *GRN* than *C9orf72* mutation carriers. Patients with heterozygous *GRN* mutations also display significant lysosomal dysfunction [87,88], which could exacerbate chitotriosidase release into CSF. Plasma [35] and CSF [89,90] chitotriosidase are highly elevated in the lysosomal storage disorder Gaucher's disease, where macrophages are chronically activated [35,91] and plasma levels are already used for monitoring treatment response [92]. Serum YKL-40 levels are also raised (and serum GRN levels are reduced) in Gaucher's disease, and recombinant GRN reduces serum YKL-40 levels in *GRN* knockout mice and in fibroblasts from patients with Gaucher's disease [91]. This strengthens the evidence for a link between GRN haploinsufficiency, glial activation, and lysosomal dysfunction, which could be detectable at an early stage, and reversible, in *GRN* mutation carriers.

The high YKL-40 levels observed in *MAPT* carriers are consistent with elevated CSF YKL-40 levels in FTLD-tau [43,49] and colocalization of activated astrocytes with tau oligomers in P301S *MAPT* mouse models [26]. There are also many activated microglia surrounding phosphorylated-tau positive neurons in *MAPT* P301S mice [8] or patients with P301S mutations [9] and pronounced frontotemporal microglial activation in *MAPT* mutation carriers [10]. This suggests that certain FLTD-tau pathologies, as well as tau in AD, may promote YKL-40 release. CSF chitotriosidase levels were also slightly raised in *MAPT* carriers but this did not reach significance compared with controls. This may have been due to the small group size, or perhaps there is greater astrocytosis than microglial activation associated with certain *MAPT* mutations.

In order to explore further how biomarkers of glial activation link to neurodegeneration, we examined relationships between YKL-40 and chitotriosidase levels and CSF neurodegenerative biomarkers that are used in clinical practice and which reflect neuronal injury and tau pathology (T-tau and P-tau) and amyloid pathology (Aβ42). Overall, both YKL-40 and chitotriosidase levels were positively associated with T-tau and P-tau levels in FTD, but this association only reached significance in certain clinical subgroups. For T-tau, there was a significant association with YKL-40 in bvFTD and nfvPPA and with chitotriosidase in bvFTD and lvPPA. For P-tau, there as a significant association with YKL-40 in bvFTD and lvPPA and with chitotriosidase in lvPPA. There was a small positive association between Aβ42 levels and YKL-40 in lvPPA, and although most subgroups seemed to have a negative association between Aβ42 and chitotriosidase, none reached significance. This variation in the strength of association between biomarkers may be explained by underlying pathologies (different FTLD subtypes or AD) being associated with varying degrees of glial activation, neurodegeneration and tau pathology, or differences in clinical subgroup sizes. Positive associations between levels of CSF YKL-40 or chitotriosidase and neurodegenerative biomarkers have been identified in many studies of typical AD, particularly for T-tau [42,47,58,65–68,93] and P-tau [42,58,65,66,68,69,93,94]. Consistent with this, there was a strong association between levels of P-tau and both glia-derived proteins in our lvPPA subgroup, where most individuals had AD-like biomarkers and high levels of both YKL-40 and chitotriosidase, suggestive of significant hyperphosphorylated tau pathology and glial activation. Our results are consistent with strong associations between T-tau and YKL-40 identified in other studies of FTD [49,58], but to our knowledge no studies have explored associations between chitotriosidase and neurodegenerative biomarkers in FTD. Our findings suggest that chitotriosidase release may be similarly linked to neurodegeneration in FTD.

In individuals with FTD but AD-like CSF (AD biomarker-positive subgroup), there were positive slopes for the association between YKL-40 and both T-tau and P-tau levels, but neither association reached significance, and chitotriosidase levels were not significantly associated with any neurodegenerative

biomarker. This contrasts with the strong associations between sTREM2 and both T-tau and P-tau levels in this group found previously [37], and in studies of amnestic AD. However, levels of YKL-40 and chitotriosidase were very high in most individuals within this subgroup, so a lack of variability combined with a relatively small sample size may have hampered our ability to detect weak associations between biomarkers. It is unclear why there was a positive association between Aβ42 and YKL-40 in this subgroup, although others have shown a similar association [47,66] or negative [94] or no significant [42] association with Aβ42 in AD. In individuals with likely FTLD (AD biomarker-negative subgroup) there was a significant positive association between YKL-40 and both T-tau and P-tau levels and a trend towards a positive association between chitotriosidase and T-tau levels. This suggests that glial activation may correlate with the degree of neuronal injury, and perhaps also tau pathology, in individuals with FTLD, supporting histopathological studies showing pronounced astrocytosis and microgliosis in FTLD, particularly tauopathies [9,10,26,28,95].

We also analysed associations between YKL-40 and chitotriosidase levels in our cohort, which to our knowledge has not been explored directly in FTD previously, although these seem to correlate moderately in AD [74] and in a combined familial MND and FTD cohort [48]. There was a strong positive association between levels of both proteins in FTD overall, and in most clinical subgroups, although this reached significance only in bvFTD and nfvPPA. Levels of YKL-40 and chitotriosidase were also highly associated in the AD biomarker-negative subgroup, but this did not reach significance in the AD biomarker-positive subgroup, again likely due to high levels of both proteins in most individuals and smaller sample size. These results suggest that astrocytic and microglial activation arise in tandem in FTD syndromes due to FTLD and perhaps AD pathology.

Finally, we examined relationships between CSF YKL-40 and chitotriosidase levels and relevant clinical parameters such as age, disease duration and sex, which could independently affect glial activation. Age was strongly associated with YKL-40 levels in the whole cohort and in the FTD group, consistent

with previous studies of YKL-40 in AD [47,61,68] and FTD [43,45,49], and a strong association between sTREM2 and age in FTD [37]. This may reflect increased glial activation associated with aging, especially within the context of neurodegeneration, and emphasises the importance of future studies of glia-derived biomarkers in neurodegenerative disease cohorts exploring associations with age and, where applicable, adjusting group comparisons for age. There was no significant association between age and chitotriosidase levels in any group. In MND, plasma chitotriosidase activity was not associated with age [57], and most studies of CSF chitotriosidase in AD and FTD have found no association with age [48,59,74]. It is unclear why this differs from YKL-40, but perhaps age has less of an influence on microglial chitotriosidase release. We found no association between either YKL-40 or chitotriosidase and disease duration in FTD, in contrast to sTREM2, where we previously described a negative association between CSF sTREM2 levels and disease duration [37], but consistent with a study of YKL-40 in FTD, where there was no association with disease duration [43]. However, our current data are cross sectional, so we were unable to explore longitudinal changes in YKL-40 or chitotriosidase levels to confirm whether these markers alter throughout the disease course. Lastly, we found no difference in YKL-40 or chitotriosidase levels between males and females in any group, suggesting limited influence of sex on release of these proteins into CSF in both healthy individuals and patients with FTD.

Limitations of this study include the small size of some of the subgroups, which may have limited our power to detect significant differences between groups. However, this is inherent to a rare disease such as FTD which has multiple phenotypes, and difficult to avoid when analysing biomarker levels across a broad spectrum of disease, while confining CSF collection and biomarker analysis to one site in order to minimise inter-centre variation. Our patient cohort contained both individuals with a clinical diagnosis of an FTD syndrome most likely due to FTLD (bvFTD, svPPA and nfvPPA) and those more commonly associated with AD pathology (lvPPA). The use of clinical diagnosis rather than pathological confirmation as an inclusion criterion meant a combination of different pathologies and mutations in the FTD group may have affected YKL-40 and chitotriosidase levels in this group overall. However, we

were able to dissect out differences in protein levels between broad pathological entities (FTLD versus AD) and gene mutations through stratification of the FTD group by CSF biomarker profile and through a preliminary analysis by mutation type. We also intentionally used a stringent cut-off of T-tau/Aβ42 ratio >1.0 to minimize misclassification of individuals into the wrong pathological subgroup, as employed previously [37]. In addition, all individuals with FTD were phenotyped in detail, meeting recent diagnostic criteria for bvFTD [38] or PPA [39].

Conclusions

In conclusion, we show that levels of two glia-derived proteins, YKL-40 and chitotriosidase, are higher in the CSF of individuals with a clinical diagnosis of FTD than in cognitively normal controls. However, levels are higher in individuals with an FTD syndrome due to underlying AD pathology (particularly lvPPA) than due to FTLD. We display preliminary evidence that there are mutation-specific differences in YKL-40 and chitotriosidase levels, with particularly pronounced elevations of YKL-40 and chitotriosidase in *GRN* mutation carriers, and YKL-40 in *MAPT* mutation carriers, which may remain undetected in mixed genetic FTD cohorts. As CSF YKL-40, and perhaps chitotriosidase, levels correlate with neurodegenerative biomarkers, particularly T-tau, and with each other, in individuals with likely FTLD, these proteins may reflect extensive astrocytic and microglial activation arising in tandem with neurodegeneration in individuals with FTLD.

Future studies should analyse CSF YKL-40 and chitotriosidase levels within larger cohorts of individuals with FTD, across a variety of clinical subgroups, and ideally in pathologically confirmed cases across the full spectrum of FTLD subtypes, with separate sporadic and genetic subgroups. Inclusion of a larger number of cases with mutations in *GRN*, *MAPT* and *C9orf72* would enable confirmation of our preliminary observations of higher protein levels in symptomatic *GRN* and *MAPT* mutation carriers. Assessment of levels in presymptomatic mutation carriers could establish when these change prior to expected symptom onset. Exploration of relationships between baseline and

longitudinal measurements of CSF YKL-40 and chitotriosidase levels, and other markers of the disease process (such as serum or CSF neurofilament light levels or frontal lobe atrophy rate) in individuals with FTD, and presymptomatic individuals, would be extremely valuable. This could improve understanding of how chronic neuroinflammation links to neurodegeneration, enable determination of whether these proteins can be used as biomarkers of disease stage, intensity and progression, and provide validation for their use in upcoming clinical trials.

List of abbreviations

AD: Alzheimer's disease **Aβ42**: β-amyloid(1–42) **bvFTD**: behavioural variant frontotemporal dementia **CBS**: corticobasal syndrome **CHI3L1:** chitinase-3-like-1 protein *C9orf72*: chromosome 9 open reading frame 72 (gene) **CSF**: cerebrospinal fluid **FTD**: frontotemporal dementia **FTLD**: frontotemporal lobar degeneration *GRN*: progranulin (gene) **GRN**: progranulin (protein) **IQR**: interquartile range **lvPPA**: logopenic variant primary progressive aphasia *MAPT*: microtubule associated protein tau (gene) **MND**: motor neuron disease **nfvPPA**: nonfluent variant primary progressive aphasia **PPA:** primary progressive aphasia **PPA-NOS**: primary progressive aphasia not otherwise specified **PSP**: progressive supranuclear palsy **P-tau**: tau phosphorylated at position threonine-181 **SD**: standard deviation **svPPA**: semantic variant primary progressive aphasia **TDP-43**: transactive DNA response binding protein 43 **TREM2**: triggering receptor expressed on myeloid cells 2 **sTREM2**: soluble TREM2 **T-tau**: total tau **YKL-40**: chitinase 3-like-1 protein

Statement of Ethics

The study was approved by the local NHS Research Ethics Committee and the Health Research Authority. All individuals gave informed written consent. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Author Contributions

IOCW and JDR were involved in study design. All authors were involved in data collection. IOCW, CH, and MSF were involved in data analysis. All authors were involved in drafting and critically revising the manuscript.

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Figure Titles and Legends

Fig 1. Comparison of CSF YKL-40 levels between groups and subgroups. Graphs show how CSF YKL-40 levels differ across (**a**) controls and overall FTD group; (**b**) controls and clinical FTD subgroups; (**c**) controls and genetic FTD subgroups; (**d**) controls and CSF biomarker-defined pathological subgroups. Horizontal bars show mean CSF YKL-40 levels and upper and lower 95% confidence intervals for each group; *$P<0.05$, **$P<0.01$

Fig 2. Comparison of CSF chitotriosidase levels between groups and subgroups. Graphs show how CSF Ln(chitotriosidase) levels differ across (**a**) controls and overall FTD group; (**b**) controls and clinical FTD subgroups; (**c**) controls and genetic subgroups; (**d**) controls and CSF biomarker-defined pathological subgroups. Horizontal bars show mean CSF Ln(chitotriosidase) levels and upper and lower 95% confidence intervals for each group; *$P<0.05$, **$P<0.01$

Fig. 3. Relationship between CSF YKL-40 and CSF neurodegenerative biomarker levels. Graphs show associations between CSF YKL-40 and T-tau (**a**), P-tau (**b**) and Aβ42 (**c**) levels for controls and overall FTD group, between YKL-40 levels and T-tau (**d**), P-tau (**e**) and Aβ42 (**f**) levels for controls and clinical FTD subgroups, and between YKL-40 levels and T-tau (**g**), P-tau (**h**) and Aβ42 (**i**) levels for controls and CSF biomarker-defined pathological subgroups. T-tau values were Ln transformed before analysis. Lines are group regression lines adjusted for age and sex(controls) and age, sex and disease duration (overall dementia group, clinical subgroups and biomarker-defined pathological subgroups). See main text for individual β and *P* values for each association.

Fig. 4. Relationship between CSF chitotriosidase and CSF neurodegenerative biomarker levels. Graphs show associations between CSF chitotriosidase and T-tau (**a**), P-tau (**b**) and Aβ42 (**c**) levels for controls and overall FTD group, between chitotriosidase levels and T-tau (**d**), P-tau (**e**) and Aβ42 (**f**) levels for controls and clinical FTD subgroups, and between chitotriosidase levels and T-tau (**g**), P-tau (**h**) and Aβ42 (**i**) levels for controls and CSF biomarker-defined pathological subgroups. Chitotriosidase and T-tau values were Ln transformed before analysis. Lines are group regression lines adjusted for

age and sex (controls) and age, sex and disease duration (overall dementia group, clinical subgroups and biomarker-defined pathological subgroups). See main text for individual β and *P* values for each association.

Fig. 5. Relationship between chitotriosidase and YKL-40 levels in CSF. Graphs show CSF chitotriosidase levels versus CSF YKL-40 levels for controls and overall FTD group (**a**), clinical FTD subgroups (**b**) and controls and CSF biomarker-defined pathological subgroups (**c**). Chitotriosidase and YKL-40 values were Ln transformed before analysis. Lines in (**a**) are group regression lines adjusted for age and sex (controls) and age, sex and disease duration (dementia group). Lines in (**b**) are group regression lines for clinical subgroups adjusted for age, sex and disease duration. Lines in (**c**) are group regression lines adjusted for age and sex (controls) and age, sex and disease duration (CSF biomarker-defined pathological subgroups). See main text for individual β and *P* values for each association

Fig. 6. Relationship between CSF YKL-40 or chitotriosidase levels and age at CSF. Graphs show CSF YKL-40 levels versus age in controls and overall FTD group (**a**) and clinical FTD subgroups (**b**), and CSF chitotriosidase levels versus age in controls and overall FTD group (**c**) and clinical FTD subgroups (**d**). Chitotriosidase values were Ln transformed before analysis. Lines in (**a**) and (**c**) are group regression lines adjusted for sex (controls) and sex and disease duration (dementia group). Lines in (**b**) and (**d**) are group regression lines for individual clinical subgroups adjusted for sex and disease duration. See main text for individual β and *P* values for each association.

Fig. 7. Relationship between CSF YKL-40 or chitotriosidase levels and disease duration at CSF. Graphs show CSF YKL-40 levels versus disease duration in the overall FTD group (**a**) and clinical FTD subgroups (**b**), and CSF chitotriosidase levels versus disease duration in the overall FTD group (**c**) and clinical FTD subgroups (**d**). Chitotriosidase values were Ln transformed before analysis. Lines in (**a**) to (**d**) are group regression lines adjusted for age and sex. See main text for individual β and *P* values for each association.