ORIGINAL RESEARCH

Plasma glial fibrillary acidic protein is raised in progranulin-associated frontotemporal dementia

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ABSTRACT

Background There are few validated fluid biomarkers in frontotemporal dementia (FTD). Glial fibrillary acidic protein (GFAP) is a measure of astrogliosis, a known pathological process of FTD, but has yet to be explored as potential biomarker.

Methods Plasma GFAP and neurofilament light chain (NfL) concentration were measured in 469 individuals enrolled in the Genetic FTD Initiative: 114 *C9orf72* expansion carriers (74 presymptomatic, 40 symptomatic), 119 *GRN* mutation carriers (88 presymptomatic, 31 symptomatic), 53 *MAPT* mutation carriers (34 presymptomatic, 19 symptomatic) and 183 non-carrier controls. Biomarker measures were compared between groups using linear regression models adjusted for age and sex with family membership included as random effect. Participants underwent standardised clinical assessments including the Mini-Mental State Examination (MMSE), Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale and MRI. Spearman's correlation coefficient was used to investigate the relationship of plasma GFAP to clinical and imaging measures.

Results Plasma GFAP concentration was significantly increased in symptomatic *GRN* mutation carriers (adjusted mean difference from controls 192.3 pg/mL, 95% CI 126.5 to 445.6), but not in those with *C9orf72* expansions (9.0, –61.3 to 54.6), *MAPT* mutations (12.7, –33.3 to 90.4) or the presymptomatic groups. GFAP concentration was significantly positively correlated with age in both controls and the majority of the disease groups, as well as with NfL concentration. In the presymptomatic period, higher GFAP concentrations were correlated with a lower cognitive score (MMSE) and lower brain volume, while in the symptomatic period, higher concentrations were associated with faster rates of atrophy in the temporal lobe.

Conclusions Raised GFAP concentrations appear to be unique to *GRN*-related FTD, with levels potentially increasing just prior to symptom onset, suggesting that GFAP may be an important marker of proximity to onset, and helpful for forthcoming therapeutic prevention trials.

INTRODUCTION

Frontotemporal dementia (FTD) is a progressive neurodegenerative condition with around a third of cases caused by an autosomal dominant gene mutation in progranulin (*GRN*), chromosome 9 open reading frame 72 (*C9orf72*) or microtubule-associated protein tau (*MAPT*).[1] As clinical trials in genetic FTD are fast approaching, robust biomarkers that allow accurate measurement of disease onset and progression are becoming increasingly important. In particular, many trials will focus on the presymptomatic stage of disease where neuropathological alterations are already present[2] and yet few biomarkers have been shown to be abnormal in this phase.[3–5]

Cerebrospinal fluid (CSF) or plasma/serum progranulin levels in *GRN* mutation carriers[4 6] and CSF (poly)GP dipeptide repeat concentrations in *C9orf72* expansion carriers[5 7 8] are markers of specific protein abnormalities in genetic FTD, but both are abnormal from early in the presymptomatic period (and potentially from birth). In contrast, neurofilament light chain (NfL) is a marker of neuronal death and axonal degeneration (measurable in CSF[3 9 10] as well as both plasma[11] and serum[12 13]) that is not specific to FTD[14] and has only been shown to be abnormal in the very late presymptomatic period prior to conversion to the symptomatic phase.[3] Glial fibrillary acidic protein (GFAP) is a marker of astrogliosis, the abnormal proliferation of astrocytes due to neuronal damage[15] and has previously been shown to be increased in frontal cortical tissue in FTD,[16] and raised in both the CSF and serum of patients with symptomatic FTD.[17–19] However, it has yet to be

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Table 1 Demographic, cognitive and biomarker data from study participants

		Presymptomatic mutation carriers			Symptomatic mutation carriers		
	Controls	*C9orf72*	*GRN*	*MAPT*	*C9orf72*	*GRN*	*MAPT*
Number of participants	183	74	88	34	40	31	19
Sex: number male (%)	81 (44.3)	29 (39.2)	34 (38.6)	13 (38.2)	27*† (67.5)	16 (51.6)	11 (57.9)
Median (IQR) age (years)	43.8 (36.3–55.1)	44.1 (34.4–52.9)	42.4 (34.6–52.7)	36.2* (31.6–44.9)	66.0*† (60.7–71.4)	64.3*‡ (59.9–70.2)	58.3*§ (53.9–64.3)
Mean (range) age at onset (years)	N/A	N/A	N/A	N/A	59 (34–71)	61 (49–76)	53 (37–66)
Median (IQR) disease duration (years)	N/A	N/A	N/A	N/A	5.6 (3.6–6.9)	2.6 (1.6–4.3)	4.3 (2.5–9.3)
Diagnosis	N/A	N/A	N/A	N/A	30 bvFTD, 8 ALS/FTD-ALS, 1 PPA, 1 PSP	15 bvFTD, 14 PPA, 1 CBS, 1 other	19 bvFTD
Median (range) MMSE	30 (26–30)	30 (25–30)	30 (26–30)	30 (27–30)	26*† (7–30)	22*‡ (0–29)	24*§ (6–30)
Median (range) FTLD-CDR-SOB	0.0 (0.0–3.0)	0.0 (0.0–3.0)	0.0 (0.0–2.5)	0.0 (0.0–2.5)	10.0*† (0.0–22.0)	10.5*‡ (2.0–21.0)	8.5*§ (1.0–21.0)
Median (IQR) plasma NfL (pg/mL)	9.3 (6.8–13.0)	11.3 (8.3–17.0)	9.2 (7.0–12.8)	8.4 (6.3–9.7)	46.0 (22.8–62.6)	92.7 (54.8–130.1)	20.5 (15.4–37.6)
Median (IQR) plasma GFAP (pg/mL)	105.8 (80.4–146.1)	116.1 (88.3–180.0)	113.4 (80.5–168.1)	89.1 (70.9–151.0)	165.7 (124.8–245.3)	272.2 (211.5–417.8)	123.3 (85.2–206.7)

For sex, age, MMSE and FTLD-CDR-SOB, significant differences are shown at p<0.05:
*Compared with controls.
†In C9orf72 group between symptomatic and presymptomatic carriers.
‡In GRN group between symptomatic and presymptomatic carriers.
§In MAPT group between symptomatic and presymptomatic carriers.
ALS, amyotrophic lateral sclerosis; bvFTD, behavioural varaint frontotemporal dementia; CBS, corticobasal syndrome; FTLD-CDR-SOB, Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale-Sum Of Boxes; GFAP, glial fibrillary acidic protein; MMSE, Mini-Mental State Examination; N/A, not applicable; NfL, neurofilament light chain; PPA, primary progressive aphasia; PSP, progressive supranuclear palsy.

explored using ultrasensitive blood-based assays in genetic FTD mutation carriers.

In this study, we aimed to investigate within the Genetic FTD Initiative (GENFI) cohort whether plasma GFAP was abnormal in each of the different genetic FTD groups during the symptomatic period, and whether we could detect any presymptomatic changes. We also aimed to explore the relationship of GFAP with plasma NfL, cognitive, and neuroimaging measures.

METHODS

Participants

Participants were recruited from GENFI, a natural history study of genetic FTD involving 23 research centres across Europe and Canada (www.genfi.org.uk)[2] involving symptomatic carriers of mutations in *GRN*, *MAPT* or *C9orf72*, and those at risk of carrying a mutation because a first-degree relative was a known symptomatic carrier. Four hundred sixty-nine consecutively recruited individuals from the GENFI study were included: 114 *C9orf72* expansion carriers (74 presymptomatic, 40 symptomatic), 119 *GRN* mutation carriers (88 presymptomatic, 31 symptomatic), 53 *MAPT* mutation carriers (34 presymptomatic, 19 symptomatic) and 183 non-carriers who acted as a control group. Demographic information is shown in table 1: age and sex differed significantly between groups. All people in the study underwent a clinical assessment consisting of a medical history with the participant and informant, and physical examination, with symptomatic status diagnosed by a clinician who was an expert in the FTD field[20–24] (specific diagnoses are shown in table 1). All participants underwent a standardised examination including the Mini-Mental State Examination (MMSE) and the Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale (FTLD-CDR).[25] Participants also performed three-dimensional T1-weighted MRI of the brain: 432 scans were available for cross-sectional analysis, of which a subgroup of 243 participants had a follow-up scan (on the same scanner) for analysis (mean (SD) interval 1.12 (0.29) years between baseline and follow-up). Volumetric measures of whole brain and cortical regions were calculated using a previously described method that uses the geodesic information flow (GIF) algorithm, which is based on atlas propagation and label fusion (online supplementary table 1).[3 26] An annualised longitudinal rate of atrophy was found by calculating the difference in each specific measure between the baseline and longitudinal scan and expressing it as a percentage of the baseline volume over 1 year (online supplementary table 2).

Measurement of plasma markers

Plasma was collected, processed and stored in aliquots at −80°C according to standardised procedures. Samples were measured using the multiplex Neurology 4-Plex A kit (102153, Quanterix, Lexington, USA) on the SIMOA HD-1 Analyzer following manufacturer's instructions. The lower limit of detection of the assay for GFAP and NfL were 0.221 and 0.104 pg/mL, respectively. Measurements were carried out at the same study site on consecutive days and the operator was blinded to all clinical information, including genetic status. To keep sample processing and plating consistent, plasma samples were thawed at room temperature for 2 hours and subsequently centrifuged at 10 000*g* for 5 min; 150 µL samples were aliquoted in a 96-well plate (Quanterix) and frozen at −80°C until analysis. Quality control samples had a mean intra-assay and interassay coefficient of variation of <10%.

Statistical analysis

Fisher's exact test was used to compare sex frequencies between groups. Distributions for demographic and biomarker data

were investigated graphically using histograms and quantile-quantile plots and tested for normality using the Shapiro-Wilk test. As demographic data did not follow a normal distribution, group differences for age at sample collection and FTLD-CDR-Sum Of Boxes were compared using the Kruskal-Wallis test. A linear regression adjusting for age was used to compare MMSE scores between groups. The primary analysis in the study was to investigate whether there were any differences in plasma GFAP concentration from controls in the different genetic mutation groups both symptomatically and presymptomatically, as well as between genetic groups. As biomarker values were not normally distributed, group means were compared by performing a linear mixed regression model with 95% bias-corrected bootstrapped CIs with 2000 repetitions in STATA (V.14; StataCorp, College Station, Texas, USA), adjusting for age and sex with family membership included as a random effect. Diagnostic performance of GFAP was assessed by areas under the curve (AUC) obtained by receiver operating characteristic (ROC) analyses, with optimal cut-off levels at the highest Youden's index (sensitivity+specificity−1) using GraphPad Prism (V.6; GraphPad Software, San Diego, California, USA). In order to investigate the relationship of GFAP concentration to demographic, cognitive and imaging measures as well as NfL concentrations, Spearman's correlation coefficient was used.

RESULTS

Plasma GFAP concentration

Plasma GFAP concentration was significantly higher in the symptomatic *GRN* mutation carriers compared with controls (adjusted mean difference 192.3 pg/mL, 95% CI 126.5 to 445.6), but not in either the symptomatic *C9orf72* (9.0, –61.3 to 54.6) or *MAPT* (12.7, –33.3 to 90.4) groups (figure 1, tables 1 and 2). Within the symptomatic groups, concentrations in *GRN* were significantly higher than both *C9orf72* (183.3, 106.1 to 427.2) and *MAPT* (179.6, 99.8 to 348.1) mutation carriers.

A ROC curve analysis measuring the ability of GFAP to distinguish symptomatic *GRN* mutation carriers from controls showed a sensitivity of 90.3% and specificity of 82.0% with a cut-off point of 163.2 pg/mL and an AUC of 0.90. For distinguishing symptomatic *GRN* mutation carriers from *C9orf72* mutation carriers there was a sensitivity of 71.0% and specificity of 70.0% with a cut-off point of 226.2 pg/mL and an AUC of 0.74, while for distinguishing symptomatic *GRN* mutation carriers from *MAPT* mutation carriers there was a sensitivity of 79.0% and specificity of 77.4% with a cut-off point of 209.1 pg/mL and an AUC of 0.80 (online supplementary figure 1).

In the presymptomatic groups, concentrations were not significantly increased in any of the groups compared with controls: *GRN* (14.2, −2.4 to 38.3), *C9orf72* (21.1, −18.8 to 66.5), *MAPT* (−7.0, −61.8 to 7.8) (figure 1, tables 1 and 2). There were also no differences across the presymptomatic groups.

Comparing symptomatic and presymptomatic carriers, a significantly higher concentration was also seen in the symptomatic versus the presymptomatic *GRN* mutation carriers (178.1, 114.3 to 365.2), but not in the other groups (figure 1, tables 1 and 2).

Correlation with age

GFAP concentration was significantly correlated with age at sample collection in controls (r=0.55, p<0.001), presymptomatic mutation carriers (all groups combined: r=0.53, p<0.001; *GRN*: r=0.58, p<0.001; *C9orf72*: r=0.50, p<0.001; *MAPT*: r=0.36, p=0.036) and symptomatic mutation carriers for all genetic groups together (r=0.38, p<0.001) and the *GRN* group alone (r=0.65, p<0.001) (figure 2). No significant correlation was seen for the symptomatic *C9orf72* (r=0.27, p=0.088) or *MAPT* mutation carriers (r=0.00, p=0.989).

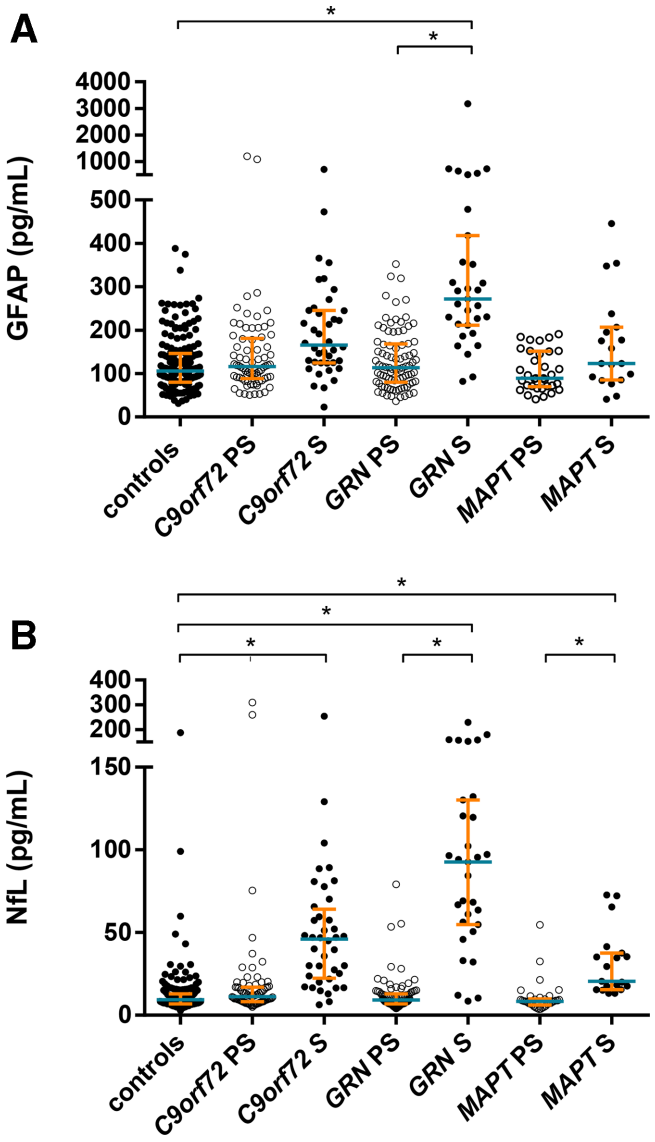

Figure 1 (A) Plasma glial fibrillary acidic protein (GFAP) and (B) plasma neurofilament light chain (NfL) concentrations (pg/mL) in control, presymptomatic and symptomatic frontotemporal dementia mutation carriers for each gene: *C9orf72, GRN* and *MAPT*. Median designated by blue line; IQRs indicated by orange error bars. *Significant differences—only differences from controls and within the same genetic group are shown on the graph. Note that 29 data points fall outside the upper or lower limit using the IQR method, with factor *k*=1.5 (ie, outside the upper limit Q3+1.5×IQR or lower limit Q1−1.5×IQR): excluding these outliers does not change the significance of the results.

Correlation with plasma NfL

Plasma NfL was increased in all three symptomatic groups compared with controls (figure 1, tables 1 and 3): *GRN* mutation carriers (adjusted mean difference 70.5 pg/mL, 95% CI 51.6 to 92.6), *C9orf72* mutation carriers (29.7, 18.7 to 47.5) and *MAPT* mutation carriers (12.6, 3.3 to 26.1). Within the symptomatic groups, concentrations in *GRN* were significantly higher than both *C9orf72* (40.7, 16.5 to 62.2) and *MAPT* (57.9, 36.8

Table 2 Adjusted mean differences in plasma GFAP concentrations between groups with 95% bias-corrected bootstrap CIs

GFAP	*C9orf72* PS	*C9orf72* S	*GRN* PS	*GRN* S	*MAPT* PS	*MAPT* S
Controls	21.1 (–18.8 to 66.5)	9.0 (–61.3 to 54.6)	14.2 (–2.4 to 38.3)	**192.3** (126.5 to 445.6)**	–7.0 (–61.8 to 7.8)	12.7 (–33.3 to 90.4)
C9orf72 PS		12.1 (–64.1 to 85.1)	–6.9 (–51.0 to 43.0)	**171.2** (88.5 to 433.7)**	–28.1 (–83.3 to 8.0)	8.4 (–87.5 to 74.5)
C9orf72 S			–5.2 (–79.4 to 39.4)	**183.3** (106.1 to 427.2)**	–16.0 (–84.3 to 32.9)	–3.7 (–104.5 to 56.1)
GRN PS				**178.1** (114.3 to 365.2)**	–21.2 (–78.3 to 1.5)	1.5 (–75.4 to 49.1)
GRN S					**–199.3** (–439.2 to –124.9)**	**–179.6** (–348.1 to –99.8)**
MAPT PS						19.6 (–30.8 to 114.4)

Significant differences in bold: **p<0.01.
GFAP, glial fibrillary acidic protein; PS, presymptomatic; S, symptomatic.

to 81.5) mutation carriers, and *C9orf72* mutation carriers were higher than *MAPT* mutation carriers (17.2, 2.9 to 35.7). Concentration was also increased in presymptomatic *C9orf72* mutation carriers compared with controls (9.0, 1.3 to 26.8), but not in the *GRN* or *MAPT* presymptomatic groups (figure 1, tables 1 and 3). Comparing symptomatic and presymptomatic carriers, a significantly higher concentration was also seen in the symptomatic versus the presymptomatic mutation carriers in each of the groups (figure 1, tables 1 and 3): *GRN* mutation carriers (70.5, 52.5 to 92.2), *C9orf72* mutation carriers (20.7, 3.2 to 36.1) and *MAPT* mutation carriers (11.7, 0.8 to 23.4).

Plasma GFAP and NfL concentrations were significantly correlated in controls (r=0.66, p<0.001), presymptomatic mutation carriers (*GRN*: r=0.66, p<0.001; *C9orf72*: r=0.75, p<0.001; *MAPT*: r=0.41, p=0.017) and symptomatic mutation carriers (*GRN*: r=0.38, p=0.036; *C9orf72*: r=0.57, p<0.001; *MAPT*: r=0.76, p<0.001).

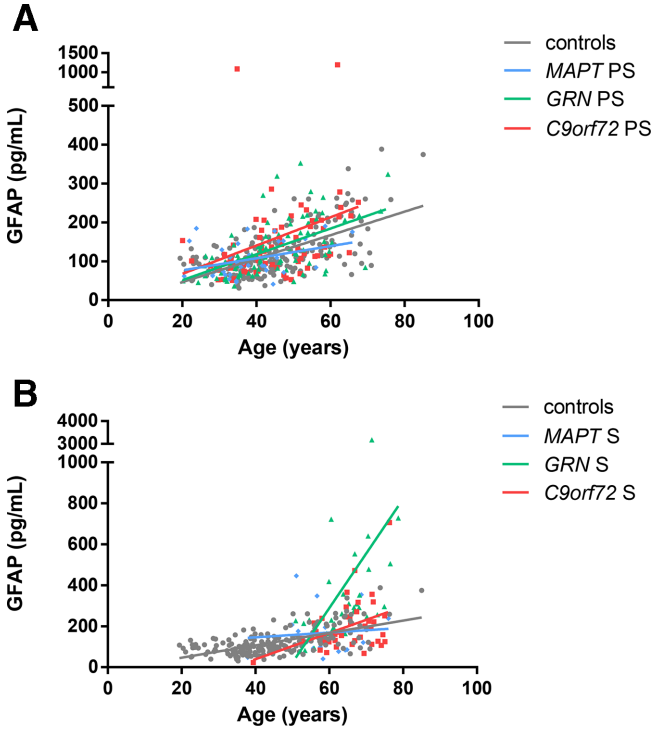

Figure 2 Correlation between plasma glial fibrillary acidic protein (GFAP) concentrations (pg/mL) and age: (A) presymptomatic and (B) symptomatic mutation carriers.

Correlation with cognitive measures

A significant negative correlation between GFAP concentrations and MMSE was seen in the presymptomatic *GRN* (r=−0.24, p=0.033) and *C9orf72* (r=−0.40, p<0.001) but not *MAPT* (r=0.05, p=0.801) mutation carriers. No significant correlation was seen during the symptomatic period in any of the genetic groups (*GRN*: r=−0.29, p=0.153; *C9orf72*: r=−0.24, p=0.146; *MAPT*: r=−0.48, p=0.080).

No significant correlations were seen between GFAP concentration and FTLD-CDR sum of boxes score in either the presymptomatic or symptomatic period in any group: *GRN*: r=−0.04, p=0.768 presymptomatic, r=0.18, p=0.409 symptomatic; *C9orf72*: r=−0.17, p=0.234 presymptomatic, r=0.21, p=0.321 symptomatic; *MAPT*: r=−0.17, p=0.446 presymptomatic, r=0.14, p=0.736 symptomatic.

Correlation with cross-sectional imaging data

A significant negative correlation was seen between GFAP concentrations and both *GRN* and *C9orf72* presymptomatic carrier brain volumes for frontal cortex (r=−0.23, p=0.039; r=−0.35, p=0.002), temporal cortex (r=−0.35, p=0.001; r=−0.27, p=0.024), cingulate cortex (r=−0.24, p=0.027; r=−0.44, p<0.001) and insular cortex (r=−0.27, p=0.016; r=−0.26, p=0.029) as well as whole brain (r=−0.45, p<0.001) and parietal cortex (r=−0.33, p=0.005) for the *C9orf72* group (online supplementary table 3). No significant correlations were seen in the presymptomatic *MAPT* mutation carrier group or any of the symptomatic genetic groups.

Correlation with longitudinal imaging data

No significant positive correlation of GFAP concentration with longitudinal rates of atrophy were seen in any of the groups except for in the temporal cortex of symptomatic *GRN* mutation carriers (r=0.66, p=0.010) (online supplementary table 4). However, within the same symptomatic *GRN* group there was also a trend in relationship between GFAP concentration and atrophy rates in the cingulate cortex (r=0.55, p=0.052).

DISCUSSION

In this study, we found that plasma GFAP concentration was significantly increased in genetic FTD but only in *GRN* mutation carriers, and not in those with *C9orf72* expansions or mutations

Table 3 Adjusted mean differences in plasma NfL concentrations between groups with 95% bias-corrected bootstrap CI

NfL	*C9orf72* PS	*C9orf72* S	*GRN* PS	*GRN* S	*MAPT* PS	*MAPT* S
Controls	**9.0*** **(1.3 to 26.8)**	**29.7**** **(18.7 to 47.5)**	0.0 (−3.1 to 3.3)	**70.5**** **(51.6 to 92.6)**	0.9 (−2.2 to 6.4)	**12.6*** **(3.3 to 26.1)**
C9orf72 PS		**20.7*** **(3.2 to 36.1)**	**−9.0*** **(−27.5 to −1.4)**	**61.4**** **(37.7 to 82.7)**	**−8.1*** **(−27.0 to −0.6)**	3.6 (−15.6 to 17.0)
C9orf72 S			**−29.8**** **(−47.7 to −18.7)**	**40.7**** **(16.5 to 62.2)**	**−28.9**** **(−46.2 to −16.9)**	**−17.2*** **(−35.7 to −2.9)**
GRN PS				**70.5**** **(52.5 to 92.2)**	−0.9 (−5.9 to 2.9)	**12.6*** **(2.9 to 25.2)**
GRN S					**−69.6**** **(−91.7 to −50.6)**	**−57.9**** **(−81.5 to −36.8)**
MAPT PS						**11.7*** **(0.8 to 23.4)**

Significant differences in bold: *p<0.05; **p<0.01.
NfL, neurofilament light chain; PS, presymptomatic; S, symptomatic.

in the *MAPT* gene. In the presymptomatic period, higher concentrations were correlated with a lower cognitive score (MMSE) and lower brain volumes (in regions characteristically affected in FTD), potentially suggesting GFAP is increased in the late presymptomatic period. In the symptomatic period, higher concentrations were associated with faster rates of atrophy, suggesting GFAP levels are associated with disease intensity, and therefore progression and survival.

GFAP is a major constituent of the astrocytic cytoskeleton and its expression pattern is highly brain-enriched.[27] Its levels increase following acute damage to astrocytes such as after a stroke[28] or traumatic brain injury,[29] but also in relation to more chronic insults, such as in neurodegeneration, when astrocytes become reactive, increasing in size and proliferating, a process called astrogliosis.[27] In neurodegeneration, increased GFAP concentrations in biofluid have been reported in Alzheimer's disease[30] (in both CSF[17 19] and serum[19]) and amyotrophic lateral sclerosis[18]. Previous studies in FTD have found increased CSF concentrations in symptomatic patients within combined clinical[17 19] and genetic cohorts[18] but have not previously found changes in blood,[19] nor investigated individual genetic groups previously. Our results suggest that there are differential increases within FTD, with concentrations being higher in people with *GRN* mutations than in other groups. *GRN* encodes the progranulin protein, which is a secreted growth factor and known to be involved in many biological processes including inflammation, wound healing and cell proliferation.[31 32] However, progranulin is also taken up by astrocytes for storage or transportation to the lysosomal compartment,[33–35] and studies of *GRN*-deficient mice have shown the presence of astrogliosis.[35–37] In vitro, progranulin seems to have a role of inactivating astrocytes with evidence that progranulin attenuates a pro-inflammatory phenotype of astrocytes.[35] This suggests that deficiency of progranulin in *GRN* mutation carriers may lead to activation of pro-inflammatory phenotypes of astrocytes and subsequent astrogliosis, with increased levels of GFAP expression. People with *GRN* mutations have evidence of astrogliosis pathologically, including within areas of white matter damage[38] (visible in a proportion of people in vivo as white matter hyperintensities on MR imaging, which have been previously shown to be unique to *GRN* mutations within familial FTD).[39] Such damage increases as the neurodegeneration progresses,[39] consistent with the pattern of increased plasma GFAP in our study. In contrast, levels were not increased in plasma in individuals with *C9orf72* expansions or *MAPT* mutations. While astrogliosis is seen in animal models and at postmortem in both *C9orf72*-related[40] and *MAPT*-related[41 42] FTD, this may well be a late feature of the disease, or the extent of astrogliosis may be less. Future work will be required to investigate this further.

It is well established that multiple biomarkers of neurodegeneration increase in concentration with age, attributed to the reduction of neural integrity in the ageing brain.[43] CSF GFAP concentrations have previously been shown to increase as one gets older,[44] with multiple studies showing proliferation of astrocytes, increased GFAP immunoreactivity and elevated levels of GFAP mRNA with age.[45–49] Consistent with this, we also found a significant positive correlation of plasma GFAP concentration with age in the majority of the groups. This highlights the importance of adjusting plasma GFAP concentrations for age in statistical analyses: symptomatic mutation carriers in the *C9orf72* and *MAPT* groups (as well as the *GRN* group) in this study showed increased levels of plasma GFAP compared with controls and their presymptomatic counterparts, but significance was lost once adjusting for age.

NfL, part of the axonal cytoskeleton, is released following cellular damage. A previous study has shown that NfL concentrations are increased in both the CSF and blood of symptomatic genetic FTD in all three mutation groups, *C9orf72*, *GRN* and *MAPT*.[3] The results in this study replicate these findings in plasma, although we also found elevated levels in presymptomatic *C9orf72* mutation carriers. In this latter group, NfL concentrations correlated negatively with MMSE ($r=-0.33$, $p=0.004$: online supplementary table 5) and with brain volumes (whole brain, $r=-0.53$, $p<0.001$ and cortical regions: frontal, −0.51, <0.001; temporal, −0.37, 0.001; parietal, −0.51, <0.001; occipital, −0.33, 0.005; cingulate, −0.49, <0.001; insula, −0.47, <0.001: online supplementary tables 6 and 7), suggesting that NfL increases particularly towards the end of the presymptomatic period with increasing neurodegeneration. Although *GRN* NfL concentration was not significantly increased presymptomatically, a similar pattern of negative correlation with brain volumes was seen in this group (in whole brain and all cortical regions except the occipital lobe, $r=-0.30$ to -0.48, $p\leq0.006$). NfL and GFAP concentrations were significantly correlated in all groups including controls, although the correlation coefficient varied from 0.38 to 0.76. A similar correlation has been shown in CSF previously.[44] As both increase with age (online supplementary table 8 for NfL correlations with age), the correlation is not unexpected, but other unexplained factors are likely to affect the different patterns within genetic FTD; interestingly,

the lowest correlation (r=0.38) was in the symptomatic *GRN* mutation carriers, suggesting that in this group astrogliosis and neurodegeneration are not so closely related.

Correlation of GFAP concentration with cognitive and imaging measures revealed a negative correlation, that is, higher concentration with a lower cognitive score and lower cross-sectional brain volumes in FTD-related regions in presymptomatic *GRN* mutation carriers. This suggests that GFAP levels start to increase as the brain starts to decrease in volume, and as cognition starts to become affected thus in the later stages of the presymptomatic period in proximity to symptom onset. This would be an important biomarker for *GRN*-related FTD, as an increase in concentration from baseline during the presymptomatic period would identify a time around the onset of neurodegeneration, and potentially a time when therapeutic intervention may be optimal. Despite the lack of a significant increase in concentration in *C9orf72* mutation carriers, a similar pattern of negative correlation with cognition and brain imaging was seen in the presymptomatic period—it would be useful in future studies to investigate the subset of *C9orf72* expansion carriers that have increased GFAP concentrations, and how they differ from those with a lower concentration. In particular, it would be helpful to compare carriers with and without concomitant ALS. We also assessed whether GFAP correlated with the rate of brain atrophy measured with longitudinal brain imaging and found a significant positive correlation only in the symptomatic *GRN* carriers (in the temporal lobe), implicating an association of GFAP levels with the intensity of the disease process, that is, how fast the disease is progressing. With longitudinal follow-up of participants, it would therefore be hypothesised that higher GFAP concentration would be associated with shorter survival in *GRN*-related FTD.

While the multicentre nature of the GENFI study allows collection of samples from a large genetic cohort of FTD worldwide, there remains a relatively small number of cases in each group (leading to low statistical power to detect differences), particularly in the symptomatic carriers, and replication in a larger dataset would be helpful. Due to the nature of the disease process, the mean age of the controls overall is lower compared with the symptomatic mutation carriers, but nonetheless the same results are found whether performing an age-adjusted comparison (as presented above in **Plasma GFAP concentration**) or when symptomatic mutation carriers are compared with an age-matched and gender-matched subset of older controls (see online supplementary figure 2). The advantage of studying levels in plasma is that blood is more easily accessible and a relatively cost-efficient way to access bodily fluids in comparison to performing a lumbar puncture; in this study, the use of the ultrasensitive SIMOA assay allowed detection at a level in blood that other assays do not. However, it will be important to study CSF levels in more detail in this group, as concentrations can differ between blood and CSF.[18] Lastly, despite significant differences between the groups, there is a substantial overlap in concentrations between carriers and controls: longitudinal study of GFAP concentration over time, particularly in participants that convert from presymptomatic to symptomatic status, will therefore be important to truly evaluate whether changes do occur towards the end of the presymptomatic period and how levels change with progression of disease.

In summary, plasma GFAP levels appear to be uniquely increased in *GRN* mutation carriers in the current study, and importantly, concentrations may well be abnormal during the late presymptomatic period, suggesting that GFAP might act as marker of proximity to symptom onset.

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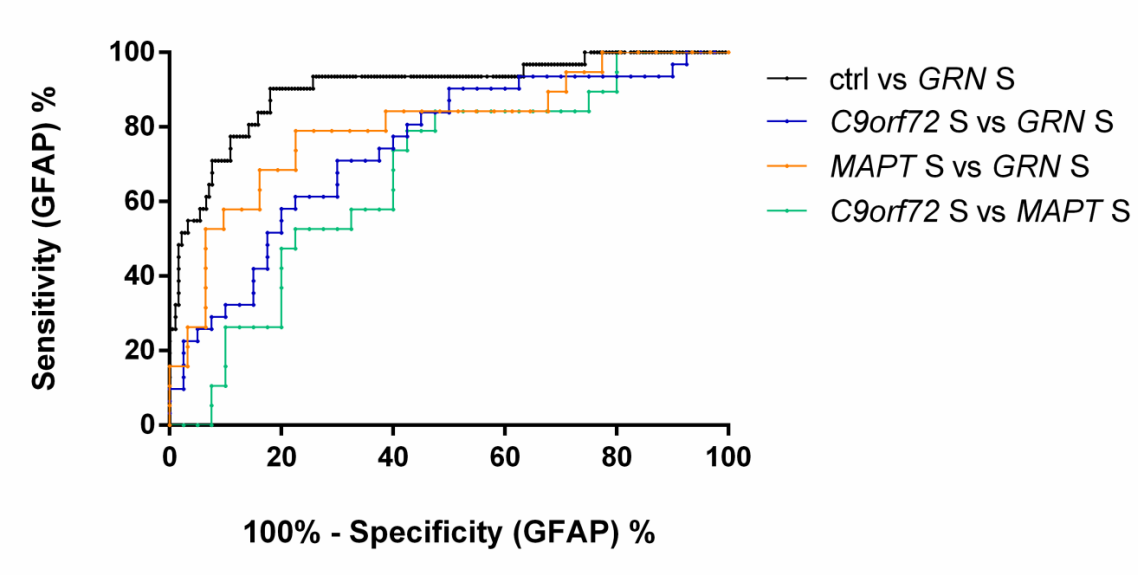
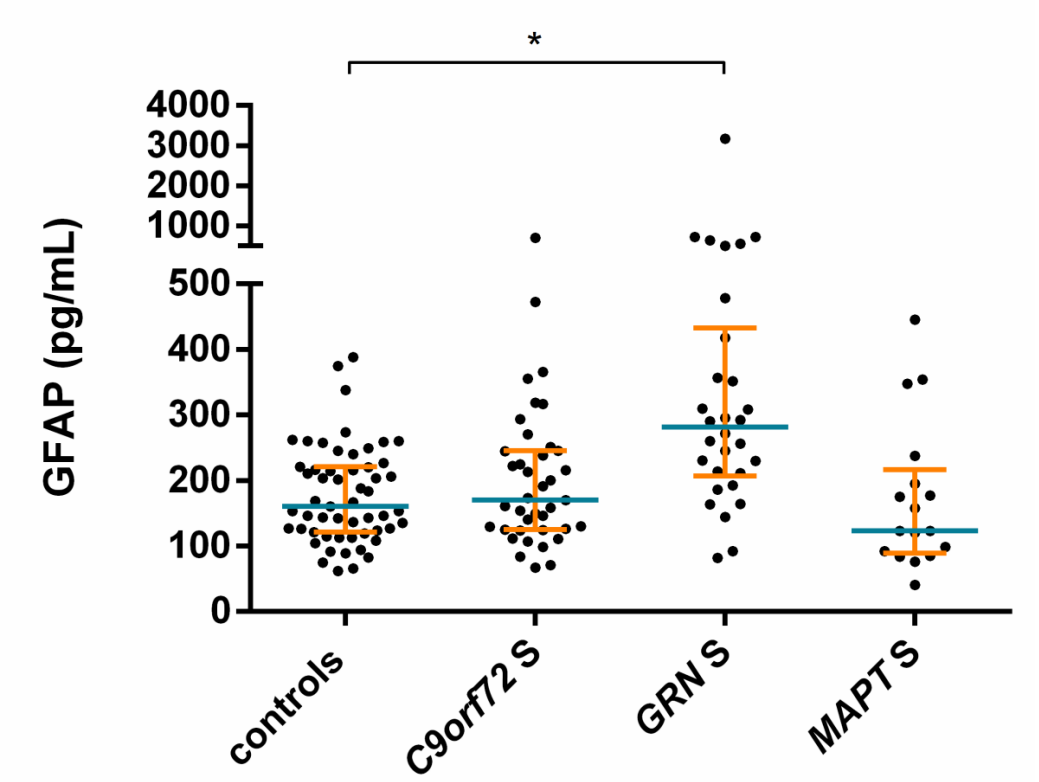
Supplementary Figure 1. Receiver operating characteristic curves showing the ability of GFAP to distinguish controls from symptomatic *GRN* mutation carriers and the symptomatic mutation carriers from one another.



Supplementary Figure 2. Plasma GFAP in older (age > 51 years) gender-matched controls (n = 55) and symptomatic FTD mutation carriers for each gene: *C9orf72* (n = 39), *GRN* (n = 30) and *MAPT* (n = 17). Median designated by blue line; interquartile ranges indicated by orange error bars. * = significant differences. Mean age [standard deviation] in years: controls = 61.4 (6.9); *C9orf72* = 66.0 (6.2); *GRN* = 64.9 (6.7) and *MAPT* = 60.9 (6.7). Median GFAP [interquartile range] (pg/mL): controls = 160.4 (121.5 - 221.3); *C9orf72* = 170.5 (125.1 - 245.4); *GRN* = 281.2 (211.5 - 417.8) and *MAPT* = 123.3 (92.5 - 195.1).



Supplementary Table 1. Summary of cross-sectional imaging data at baseline. The whole brain and six cortical regions are expressed as a percentage of total intracranial volume. Values are displayed as medians with interquartile ranges. PS – presymptomatic; S – symptomatic.

	Controls	*C9orf72* PS	*C9orf72* S	*GRN* PS	*GRN* S	*MAPT* PS	*MAPT* S
Number of participants	171	72	34	83	24	34	14
Whole brain	80.7 (79.0 - 82.5)	79.6 (76.7 - 81.4)	72.4 (69.0 - 76.3)	80.8 (79.3 - 82.5)	71.1 (69.1 - 75.3)	81.2 (79.2 - 82.1)	72.2 (71.3 - 74.4)
Frontal	12.5 (12.0 – 13.0)	12.3 (11.8 - 12.8)	10.5 (9.7 - 11.2)	12.8 (12.1 - 13.2)	10.3 (9.8 - 11.1)	12.5 (12.1 - 12.9)	11.2 (11.0 - 11.5)
Temporal	8.5 (8.2 - 8.7)	8.3 (8.1 - 8.6)	7.6 (7.1 - 8.0)	8.6 (8.2 - 8.9)	7.7 (7.2 - 8.1)	8.6 (8.2 - 8.9)	6.3 (6.1 - 7.3)
Parietal	6.6 (6.3 - 6.9)	6.4 (6.1 - 6.7)	5.7 (5.3 - 5.9)	6.8 (6.4 - 7.0)	5.7 (5.3 - 6.2)	6.6 (6.3 - 6.9)	6.0 (5.7 - 6.4)
Occipital	5.1 (4.9 - 5.5)	5.0 (4.8 - 5.3)	4.5 (4.3 - 4.9)	5.2 (5.0 - 5.4)	5.0 (4.7 - 5.2)	5.1 (4.7 - 5.4)	4.9 (4.7 - 5.1)
Cingulate	2.0 (1.9 - 2.1)	2.0 (1.9 - 2.1)	1.8 (1.7 - 1.9)	2.0 (1.9 - 2.2)	1.7 (1.7 - 1.9)	2.0 (1.9 - 2.1)	1.8 (1.7 - 1.9)
Insula	0.8 (0.7 - 0.8)	0.7 (0.7 - 0.8)	0.5 (0.5 - 0.6)	0.8 (0.7 - 0.8)	0.6 (0.5 - 0.6)	0.8 (0.7 - 0.8)	0.5 (0.5 - 0.6)

Supplementary Table 2. Summary of longitudinal imaging data. The whole brain and six cortical regions are expressed as an annualized percentage rate of atrophy. Values are displayed as medians with interquartile ranges. PS – presymptomatic; S – symptomatic.

	Controls	*C9orf72* PS	*C9orf72* S	*GRN* PS	*GRN* S	*MAPT* PS	*MAPT* S
Number of participants	102	38	15	46	14	24	4
Scan interval (years)	1.1 (1.0 - 1.2)	1.1 (1.0 - 1.2)	1.1 (0.9 - 1.2)	1.0 (1.0 - 1.1)	1.0 (1.0 - 1.1)	1.1 (1.1 - 1.1)	1.0 (1.0 - 1.4)
Whole brain	0.0 (-0.3 - 0.4)	0.5 (-0.1 - 1.0)	1.0 (-0.3 - 2.1)	0.2 (-0.2 - 0.5)	2.4 (1.2 - 3.0)	0.1 (-0.9 - 0.6)	0.7 (0.5 - 1.6)
Frontal	0.1 (-1.0 - 0.7)	0.2 (-0.6 - 1.1)	1.5 (-0.7 - 2.7)	0.2 (-0.7 - 0.6)	3.6 (0.9 - 9.1)	-0.3 (-2.3 - 1.2)	1.6 (0.6 - 4.5)
Temporal	-0.2 (-0.8 - 0.5)	0.5 (-0.4 - 1.1)	0.2 (-0.8 - 2.5)	0.2 (-0.5 - 0.7)	2.3 (1.6 - 4.4)	0.1 (-1.6 - 0.5)	0.6 (-0.2 - 1.5)
Parietal	0.1 (-1.0 - 1.2)	-0.1 (-1.3 - 1.3)	0.8 (-1.6 - 4.3)	0.5 (-0.3 - 1.4)	3.6 (0.9 - 5.6)	0.0 (-0.7 - 1.0)	0.6 (-0.1 - 3.3)
Occipital	0.6 (-0.7 - 1.7)	0.7 (-0.9 - 2.4)	1.0 (-2.4 - 2.2)	0.6 (-0.6 - 1.7)	1.1 (0.4 - 2.6)	-0.3 (-1.0 - 1.1)	1.8 (0.1 - 2.9)
Cingulate	0.2 (-1.0 - 0.8)	0.9 (-0.4 - 2.1)	0.8 (-0.5 - 2.2)	0.5 (-0.4 - 1.2)	3.4 (1.7 - 4.7)	-0.7 (-2.4 - 1.6)	2.5 (2.0 - 2.7)
Insula	0.0 (-1.4 - 1.3)	0.7 (-1.2 - 1.6)	-0.3 (-2.6 - 2.4)	0.6 (-0.4 - 1.7)	7.0 (1.7 - 10.8)	-0.1 (-1.4 - 1.8)	2.3 (1.5 - 3.5)

Supplementary Table 3. Spearman's correlation coefficients (r, and p value) assessing the relationship between plasma GFAP concentration and baseline brain and cortical regional volumes. PS – presymptomatic; S – symptomatic. Significant negative correlations shown in bold.

		***C9orf72* PS**	***C9orf72* S**	***GRN* PS**	***GRN* S**	***MAPT* PS**	***MAPT* S**
Whole brain	**r**	**-0.45**	-0.12	-0.21	-0.10	0.13	-0.23
	p-value	**<0.001**	0.507	0.054	0.630	0.465	0.436
Frontal	**r**	**-0.35**	-0.23	**-0.23**	-0.24	-0.09	-0.23
	p-value	**0.002**	0.183	**0.039**	0.255	0.606	0.427
Temporal	**r**	**-0.27**	-0.12	**-0.35**	-0.36	-0.05	-0.17
	p-value	**0.024**	0.489	**0.001**	0.082	0.782	0.563
Parietal	**r**	**-0.33**	-0.12	-0.09	-0.17	0.01	-0.47
	p-value	**0.005**	0.513	0.440	0.433	0.953	0.088
Occipital	**r**	-0.15	-0.15	0.00	-0.28	0.05	-0.43
	p-value	0.215	0.408	0.992	0.188	0.803	0.126
Cingulate	**r**	**-0.44**	-0.16	**-0.24**	-0.21	0.23	-0.27
	p-value	**<0.001**	0.383	**0.027**	0.318	0.200	0.358
Insula	**r**	**-0.26**	-0.28	**-0.27**	-0.18	0.06	-0.02
	p-value	**0.029**	0.105	**0.016**	0.402	0.719	0.958

Supplementary Table 4. Spearman's correlation coefficients (r, and p value) assessing the relationship between plasma GFAP concentration and longitudinal brain and cortical regional rates of atrophy. PS – presymptomatic; S – symptomatic. Significant positive correlations shown in bold.

		***C9orf72* PS**	***C9orf72* S**	***GRN* PS**	***GRN* S**	***MAPT* PS**	***MAPT* S**
Whole brain	**r**	0.03	0.05	0.02	0.07	-0.02	-0.40
	p-value	0.856	0.870	0.906	0.805	0.917	0.600
Frontal	**r**	-0.03	0.17	-0.08	0.20	-0.05	0.80
	p-value	0.870	0.541	0.608	0.493	0.828	0.200
Temporal	**r**	-0.26	0.03	0.13	**0.66**	0.12	-0.40
	p-value	0.121	0.920	0.373	**0.010**	0.577	0.600
Parietal	**r**	-0.09	-0.07	0.05	0.40	-0.17	0.40
	p-value	0.597	0.810	0.762	0.159	0.428	0.600
Occipital	**r**	0.14	-0.07	0.10	0.24	-0.54	0.80
	p-value	0.395	0.800	0.503	0.401	0.007	0.200
Cingulate	**r**	0.17	0.07	-0.17	0.55	-0.07	-0.40
	p-value	0.300	0.810	0.264	0.052	0.735	0.600
Insula	**r**	0.06	-0.03	0.15	0.18	-0.04	-0.80
	p-value	0.711	0.910	0.328	0.533	0.853	0.200

Supplementary Table 5. Spearman's correlation coefficients (r, and p value) assessing the relationship between plasma NfL concentration and both MMSE and FTLD-CDR sum of boxes. PS – presymptomatic; S – symptomatic. Significant negative correlations shown in bold.

		***C9orf72* PS**	***C9orf72* S**	***GRN* PS**	***GRN* S**	***MAPT* PS**	***MAPT* S**
MMSE	**r**	**-0.33**	**-0.35**	-0.12	-0.35	0.18	-0.45
	p-value	**0.004**	**0.031**	0.124	0.084	0.323	0.105
FTLD-CDR	**r**	-0.06	0.10	0.19	0.32	0.25	**0.79**
	p-value	0.676	0.648	0.134	0.126	0.248	**0.021**

Supplementary Table 6. Spearman's correlation coefficients (r, and p value) assessing the relationship between plasma NfL concentration and baseline brain and cortical regional volumes. PS – presymptomatic; S – symptomatic. Significant negative correlations shown in bold.

		***C9orf72* PS**	***C9orf72* S**	***GRN* PS**	***GRN* S**	***MAPT* PS**	***MAPT* S**
Whole brain	**r**	**-0.53**	-0.03	**-0.35**	-0.25	-0.13	-0.19
	p-value	**<0.001**	0.855	**0.001**	0.243	0.478	0.523
Frontal	**r**	**-0.51**	-0.23	**-0.30**	**-0.46**	-0.21	-0.18
	p-value	**<0.001**	0.181	**0.006**	**0.024**	0.237	0.533
Temporal	**r**	**-0.37**	0.02	**-0.48**	0.11	-0.14	-0.22
	p-value	**0.001**	0.900	**<0.001**	0.613	0.425	0.446
Parietal	**r**	**-0.51**	-0.07	**-0.36**	-0.17	-0.07	-0.33
	p-value	**<0.001**	0.685	**0.001**	0.416	0.686	0.246
Occipital	**r**	**-0.33**	0.03	-0.09	0.02	-0.16	-0.13
	p-value	**0.005**	0.860	0.423	0.942	0.380	0.659
Cingulate	**r**	**-0.49**	0.02	**-0.37**	-0.12	-0.29	-0.30
	p-value	**<0.001**	0.909	**0.001**	0.582	0.096	0.303
Insula	**r**	**-0.47**	-0.08	**-0.32**	**-0.50**	-0.13	0.06
	p-value	**<0.001**	0.634	**0.004**	**0.012**	0.456	0.840

Supplementary Table 7. Spearman's correlation coefficients (r, and p value) assessing the relationship between plasma NfL concentration and longitudinal brain and cortical regional rates of atrophy. PS – presymptomatic; S – symptomatic. Significant positive correlations shown in bold.

		***C9orf72* PS**	***C9orf72* S**	***GRN* PS**	***GRN* S**	***MAPT* PS**	***MAPT* S**
Whole brain	**r**	0.17	-0.28	0.16	**0.63**	-0.10	0.20
	p-value	0.317	0.315	0.280	**0.016**	0.639	0.800
Frontal	**r**	-0.10	0.17	-0.15	**0.79**	-0.27	**1.00**
	p-value	0.545	0.541	0.311	**0.001**	0.210	**<0.001**
Temporal	**r**	-0.17	-0.01	0.29	**0.54**	-0.08	0.20
	p-value	0.317	0.980	0.051	**0.047**	0.722	0.800
Parietal	**r**	-0.09	0.05	0.21	0.41	-0.21	0.80
	p-value	0.607	0.870	0.159	0.144	0.314	0.200
Occipital	**r**	-0.12	0.15	0.00	0.08	-0.35	**1.00**
	p-value	0.479	0.585	0.991	0.782	0.099	**<0.001**
Cingulate	**r**	0.15	-0.23	0.01	0.41	-0.15	0.20
	p-value	0.354	0.413	0.960	0.162	0.498	0.800
Insula	**r**	-0.01	-0.08	0.25	**0.63**	-0.07	-0.40
	p-value	0.934	0.791	0.099	**0.016**	0.731	0.600

Supplementary Table 8. Spearman's correlation coefficients (r, and p value) assessing the relationship between plasma NfL concentration and age. Significant correlations shown in bold.

		Controls	*C9orf72* PS	*C9orf72* S	*GRN* PS	*GRN* S	*MAPT* PS	*MAPT* S
Age	**r**	**0.64**	**0.64**	0.05	**0.74**	0.10	**0.60**	-0.31
	p-value	**<0.001**	**<0.001**	0.767	**<0.001**	0.596	**<0.001**	0.201