

# Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia

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## Introduction

- Clinical trials in dementia would benefit from effective blood-based measures of disease state or progression. However, we currently lack reliable blood-based biomarkers in frontotemporal dementia (FTD).
- Neurofilament is a protein that maintains the structural integrity of axons and has 3 subunits: heavy chain, medium chain and light chain (NfL). Cerebrospinal fluid (CSF) NfL concentrations increase with axonal damage in multiple sclerosis and may correlate with disease severity and progression in motor neuron disease. CSF NfL concentrations correlate with disease severity in FTD, but blood-based (serum) markers would be more useful as they are less invasive to obtain.
- We developed a novel ultrasensitive assay that detects NfL concentrations at very low levels in serum. We compared serum NfL concentrations between a group of patients with FTD (including a variety of clinical and genetic subgroups) and healthy controls and analysed the relationship between serum NfL concentrations and a variety of neuroimaging and neuropsychological measures of disease.

## Methods

- We collected serum samples from 67 patients with FTD, consecutively recruited from the University College London FTD study, 7 patients with logopenic variant primary progressive aphasia (lvPPA) and 28 healthy controls matched for age and gender (Table 1). 24/67 FTD subjects carried a pathogenic mutation in either *C9orf72* (n=9; 8 bvFTD and 1 nfvPPA), microtubule-associated protein tau, *MAPT* (n=11; all bvFTD) or progranulin, *GRN* (n=4; 1 bvFTD, 1 nfvPPA, 2 PPA-NOS). No mutations were found in the other participants.

Table 1

Disease group	Controls	Total FTD	bvFTD	FTD-MND	nfvPPA	svPPA	PPA-NOS	lvPPA
Number	28	67	34	3	13	10	7	7
Age, years [mean (SD)]	63.9 (7.2)	64.5 (7.9)	63.0 (8.3)	65.0 (0.3)	67.5 (9.7)	66.2 (6.4)	63.9 (5.2)	65.6 (5.9)
Male gender [%]	46.4	61.2	73.5	66.7	23.1	60.0	71.4	71.4
Disease duration, years [mean (SD)]	N/A	5.5 (3.7)	6.2 (4.6)	6.0 (4.6)	3.8 (1.5)	6.0 (2.1)	4.5 (2.5)	6.4 (2.9)
Serum NfL, pg/ml [mean (SD)]	19.6 (8.2)	77.9 (51.3)	57.8 (33.1)	195.0 (69.9)	82.5 (33.8)	95.9 (33.0)	91.2 (86.6)	49.5 (19.4)

Table 1. Demographic characteristics of the study participants. Total FTD cases do not include lvPPA. bvFTD = behavioural variant FTD, FTD-MND = FTD with motor neurone disease, PPA = primary progressive aphasia, nfvPPA = nonfluent variant PPA, svPPA = semantic variant PPA, lvPPA = logopenic variant PPA, PPA-NOS = PPA not otherwise specified. NfL = neurofilament light chain; SD = standard deviation.

- Serum NfL concentrations were determined using the NF-Light kit, transferred onto the Simoa platform, using a homebrew kit (Quanterix Corp, Boston, MA, USA). Protocol details can be found in the Simoa Homebrew Assay Development Guide (Quanterix). The lower limit of serum NfL concentration quantification was 0.26 pg/ml.
- A proportion of FTD patients had baseline (n=46/67) and follow up (n=29/46) volumetric T1 brain magnetic resonance imaging on a 3T Siemens Trio scanner and baseline (n=47/67) and follow up (n=29/47) neuropsychological tests: Weschler Abbreviated Scale of Intelligence (WASI) Vocabulary, Block Design, Similarities and Matrices subtests, the Recognition Memory Tests for Faces and Words, the Graded Naming Test, the Graded Difficulty Calculation Test, the D-KEFS Color-Word Interference Test and the Mini-Mental State Examination. Mean (standard deviation, SD) interval between serum sampling and baseline assessments was 0.0 (0.2) years. Mean (SD) interval between baseline and follow up assessments was 1.1 (0.2) years.
- Whole brain volumes were measured using a semi-automated segmentation method, with annualized whole brain atrophy rates calculated using the boundary shift integral. Individual lobar cortical volumes were measured using a multi-atlas segmentation propagation approach following the brainCOLOR protocol, combining regions of interest to calculate grey matter volumes for each lobe. Annualized lobar atrophy rates were calculated using the differences in volumes between baseline and follow up scans, and dividing by the interval between scans.

## Results

### Clinical groups

- Mean serum NfL concentrations were higher in patients with FTD than controls (Table 1;  $p < 0.001$ ). Concentrations were significantly higher in bvFTD cases than controls ( $p < 0.001$ ) and in both nfvPPA ( $p < 0.001$ ) and svPPA ( $p = 0.001$ ) cases compared with controls (Figure 1). There was a trend for a higher concentration in svPPA compared with bvFTD cases (mean difference = 38.1 pg/ml;  $p = 0.070$ ). Concentrations were higher in svPPA than lvPPA cases (mean difference = 46.3 pg/ml;  $p = 0.032$ ).

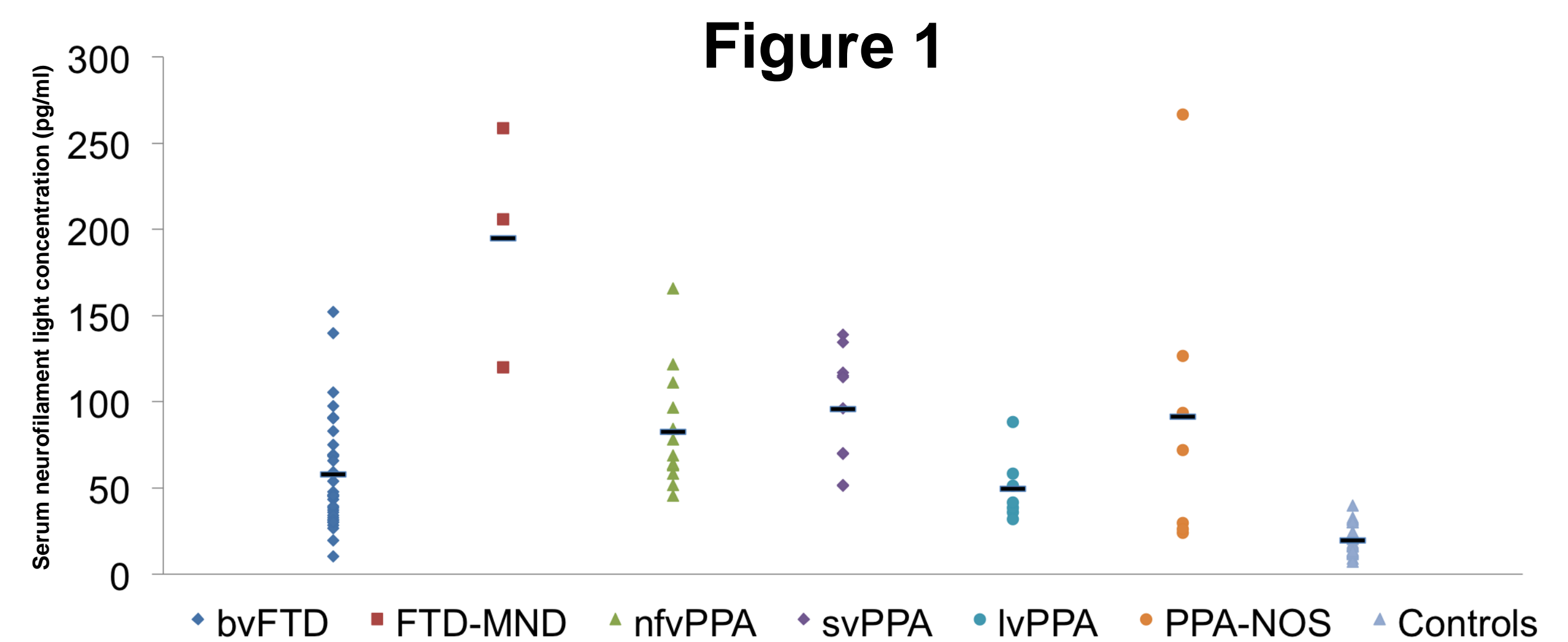


Figure 1. Serum neurofilament light chain (NfL) concentrations in participants by clinical diagnosis. Horizontal black bars represent group mean serum NfL concentration.

### Genetic groups

- Serum NfL concentrations were higher than controls in each of the genetic subgroups (Figure 2). Mean (SD) levels: 138.5 (103.3) pg/ml in *GRN* cases, 79.2 (48.2) pg/ml in *C9orf72* cases and 40.5 (20.9) pg/ml in *MAPT* cases. However, only the *MAPT* subgroup (mean difference from controls = 20.8, 95% CI = 1.4, 40.3;  $p = 0.035$ ) and the *C9orf72* subgroup (mean difference from controls = 59.5, 95% CI = 8.0, 111.0;  $p = 0.025$ ) were significantly different from controls. There was no significant difference between genetic groups.

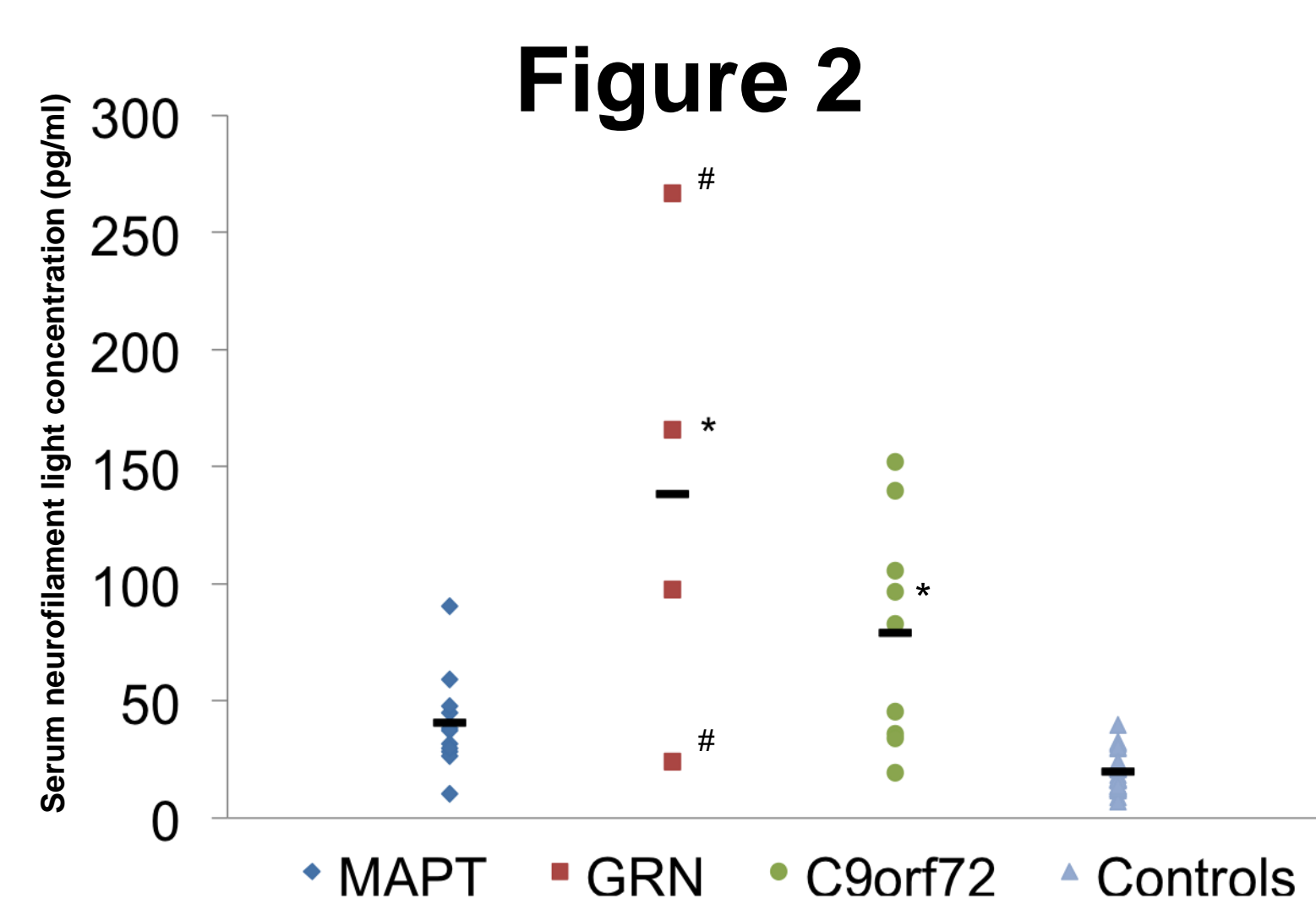


Figure 2. Serum neurofilament light chain (NfL) concentrations in participants by genetic status. All patients have bvFTD except for \*with nfvPPA and #with PPA-NOS. Horizontal black bars represent group mean serum NfL concentration.

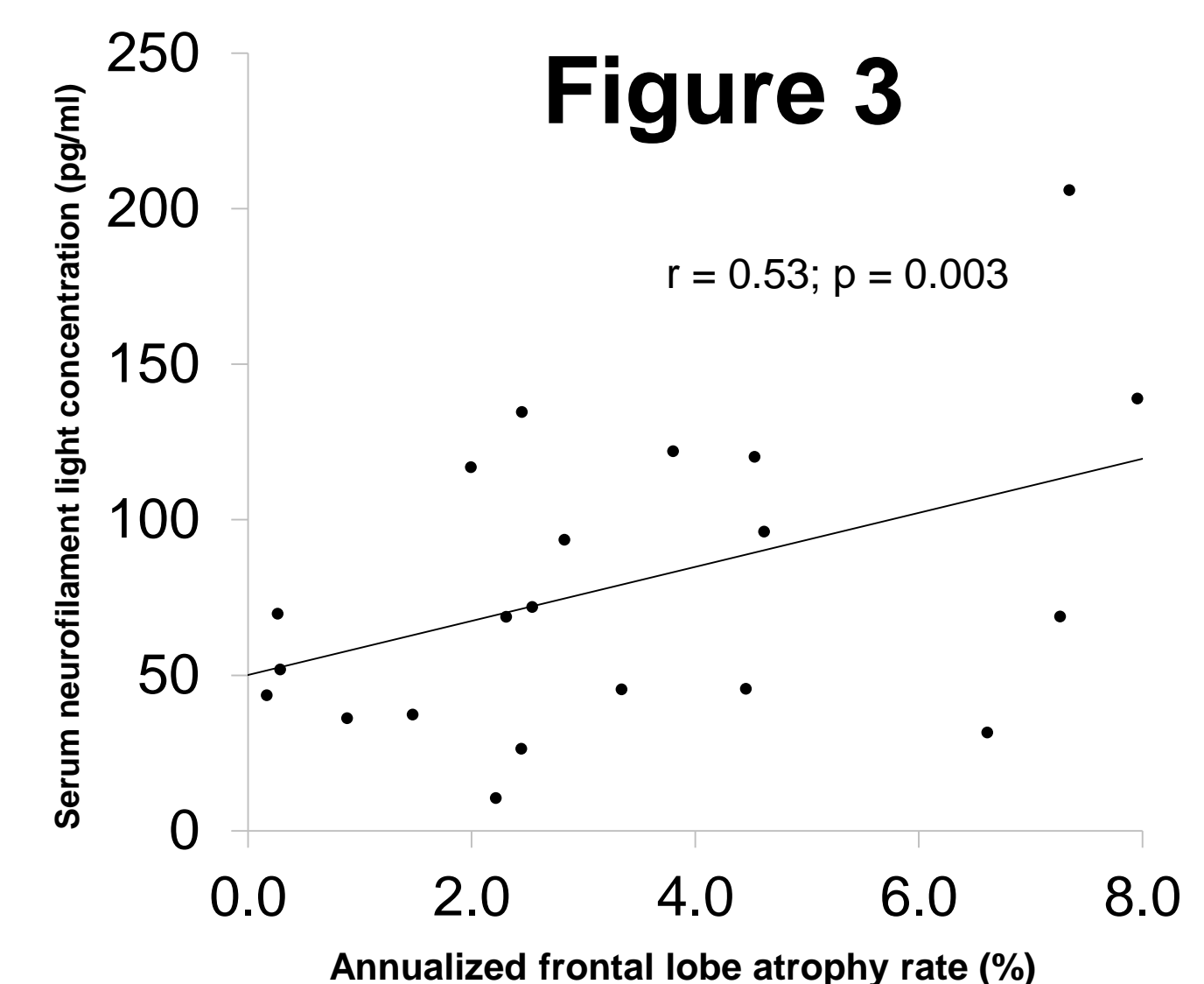


Figure 3. Serum neurofilament light chain concentration is correlated with the rate of frontal atrophy in FTD. Points indicate individual patient values and straight line indicates line of best fit from a linear regression model.

### Disease correlations

- Serum NfL concentrations were correlated with rates of whole brain ( $r = 0.46$ ,  $p = 0.01$ ), frontal lobe ( $r = 0.53$ ,  $p = 0.003$ ) and parietal lobe ( $r = 0.38$ ,  $p = 0.04$ ) atrophy, although not with other lobar atrophy rates. However, only the frontal lobe rate of atrophy survived correction for multiple comparisons (Figure 3). There were no significant correlations with baseline brain volumes. Serum NfL concentrations were correlated with baseline measures of executive dysfunction [WASI similarities ( $r = -0.32$ ,  $p = 0.03$ ) and D-KEFS Color-Word Interference ink colour naming task ( $r = -0.35$ ,  $p = 0.03$ )] but not with other baseline neuropsychological tests, nor with longitudinal changes in psychometric measures. No neuropsychological measures survived correction for multiple comparisons.

## Conclusions

- Using a novel ultrasensitive immunoassay, we show that serum NfL concentrations are raised in FTD and that higher concentrations are associated with a faster rate of frontal lobe atrophy. Within the FTD subtypes, there was a tendency for patients with probable TDP-43 pathology (svPPA and FTD-MND clinically, *GRN* and *C9orf72* mutations genetically) to have higher levels compared with patients with tau pathology (*MAPT* mutations) who tend to have a slower disease course.
- This study suggests that serum NfL concentrations reflect the intensity of disease in FTD and could be used as a predictor of disease progression.
- As blood sampling is less invasive than lumbar puncture, serum NfL concentration may prove to be a useful outcome measure for use in future clinical trials in FTD.

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