Serum neurofilament light chain in genetic frontotemporal dementia: a longitudinal, multicentre cohort study

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Summary

Background Neurofilament light chain (NfL) is a promising blood biomarker in genetic frontotemporal dementia, with elevated concentrations in symptomatic carriers of mutations in *GRN*, *C9orf72*, and *MAPT*. A better understanding of NfL dynamics is essential for upcoming therapeutic trials. We aimed to study longitudinal NfL trajectories in people with presymptomatic and symptomatic genetic frontotemporal dementia.

Methods We recruited participants from 14 centres collaborating in the Genetic Frontotemporal Dementia Initiative (GENFI), which is a multicentre cohort study of families with genetic frontotemporal dementia done across Europe and Canada. Eligible participants (aged ≥18 years) either had frontotemporal dementia due to a pathogenic mutation in *GRN*, *C9orf72*, or *MAPT* (symptomatic mutation carriers) or were healthy at-risk first-degree relatives (either presymptomatic mutation carriers or non-carriers), and had at least two serum samples with a time interval of 6 months or more. Participants were excluded if they had neurological comorbidities that were likely to affect NfL, including cerebrovascular events. We measured NfL longitudinally in serum samples collected between June 8, 2012, and Dec 8, 2017, through follow-up visits annually or every 2 years, which also included MRI and neuropsychological assessments. Using mixed-effects models, we analysed NfL changes over time and correlated them with longitudinal imaging and clinical parameters, controlling for age, sex, and study site. The primary outcome was the course of NfL over time in the various stages of genetic frontotemporal dementia.

Findings We included 59 symptomatic carriers and 149 presymptomatic carriers of a mutation in *GRN*, *C9orf72*, or *MAPT*, and 127 non-carriers. Nine presymptomatic carriers became symptomatic during follow-up (so-called converters). Baseline NfL was elevated in symptomatic carriers (median 52 pg/mL [IQR 24–69]) compared with presymptomatic carriers (9 pg/mL [6–13]; p<0·0001) and non-carriers (8 pg/mL [6–11]; p<0·0001), and was higher in converters than in non-converting carriers (19 pg/mL [17–28] *vs* 8 pg/mL [6–11]; p=0·0007; adjusted for age). During follow-up, NfL increased in converters (b=0·097 [SE 0·018]; p<0·0001). In symptomatic mutation carriers overall, NfL did not change during follow-up (b=0·017 [SE 0·010]; p=0·101) and remained elevated. Rates of NfL change over time were associated with rate of decline in Mini Mental State Examination (b=–94·7 [SE 33·9]; p=0·003) and atrophy rate in several grey matter regions, but not with change in Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale score (b=–3·46 [SE 46·3]; p=0·941).

Interpretation Our findings show the value of blood NfL as a disease progression biomarker in genetic frontotemporal dementia and suggest that longitudinal NfL measurements could identify mutation carriers approaching symptom onset and capture rates of brain atrophy. The characterisation of NfL over the course of disease provides valuable information for its use as a treatment effect marker.

Funding ZonMw and the Bluefield project.

Introduction

Frontotemporal dementia is a common cause of young-onset dementia and is characterised by progressive behavioural or language changes, or both.[1,2] Autosomal dominant inheritance is present in 20–30% of cases, most commonly owing to mutations in granulin (*GRN*), chromosome 9 open reading frame 72 (*C9orf72*), or microtubule-associated protein tau (*MAPT*).[3] With upcoming therapeutic trials, biomarkers are needed to identify the appropriate time to start treatment, probably in the preclinical stage, and as surrogate endpoints to measure treatment effect.

Neurofilament light chain (NfL), a constituent of the axonal cytoskeleton, is a promising diagnostic and prognostic blood biomarker in genetic frontotemporal dementia, with low concentrations in presymptomatic mutation

Lancet Neurol 2019; 18: 1103–11

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Research in context

Evidence before this study

We searched PubMed from database inception to May 16, 2019, for longitudinal studies of blood neurofilament light chain (NfL) in dementias using the following terms: "dementia" OR "neurodegenerative" OR "frontotemporal" OR "Pick" OR "Alzheimer" OR "Parkinson" OR "Huntington" OR "amyotrophic lateral sclerosis" AND "neurofilament" AND "blood" OR "serum" OR "plasma" AND "longitudinal" OR "repeated" OR "follow up". Although several cross-sectional studies reported elevated NfL concentrations in genetic frontotemporal dementia, we found no longitudinal NfL studies in patients with genetic frontotemporal dementia. A recent large longitudinal study of familial Alzheimer's disease reported an increased rate of NfL change more than 15 years before symptom onset, whereas a smaller study of familial amyotrophic lateral sclerosis showed NfL increases up to 12 months before symptom onset. Longitudinal NfL studies in the symptomatic stage of sporadic neurodegenerative disorders have shown inconsistent results.

Added value of this study

This longitudinal study of blood-derived NfL in a large cohort (n=335) of presymptomatic and symptomatic frontotemporal dementia mutation carriers (*GRN*, *C9orf72*, and *MAPT*) shows stable NfL concentrations in most presymptomatic mutation carriers, a sharp increase around conversion to the symptomatic stage, and overall stable, elevated concentrations during the disease course. Nine presymptomatic mutation carriers who developed frontotemporal dementia during follow-up had elevated NfL concentrations 1–2 years before symptom onset. The rate of NfL increase over time correlated with the rate of brain atrophy in several grey matter regions.

Implications of all the available evidence

This study shows serum NfL to be an easily accessible biomarker in genetic frontotemporal dementia. The repeated measurement of NfL appears to be a robust measure to identify mutation carriers approaching symptom onset. The characterisation of NfL over the course of genetic frontotemporal dementia provides valuable information for its use as a treatment effect marker in therapeutic trials.

carriers and high concentrations in symptomatic carriers.[4–6] NfL is elevated in various other neurological diseases, probably reflecting neuroaxonal degeneration.[7] In patients with multiple sclerosis, decreases in NfL have been seen after anti-inflammatory treatment,[8] and in mouse models of Alzheimer's disease decreases were seen after inhibition of amyloid-β deposits,[9] suggesting that NfL is a dynamic marker of disease activity.

When NfL starts to increase and how NfL changes over the course of frontotemporal dementia is unknown. The Genetic Frontotemporal Dementia Initiative (GENFI), which follows-up carriers of mutations in *GRN*, *C9orf72*, and *MAPT*, provides an opportunity to prospectively study disease progression from presymptomatic to overt frontotemporal dementia and to identify biomarkers of early pathological processes.

We aimed to longitudinally measure serum NfL using an ultrasensitive single molecule array in the GENFI cohort to evaluate its temporal profile. We used brain imaging and clinical datasets to study whether NfL changes correlate with brain atrophy and clinical decline.

Methods

Study design and participants

We recruited participants from centres collaborating in GENFI, which follows patients with frontotemporal dementia due to a pathogenic mutation in *GRN*, *C9orf72*, or *MAPT* (symptomatic mutation carriers) and healthy at-risk first-degree relatives (either presymptomatic mutation carriers or non-carriers).[10] We included participants if at least two serum samples were available with a time interval of 6 months or more. Exclusion criteria were neurological comorbidities that were likely to affect NfL, including cerebrovascular events, or cognitive disorders other than frontotemporal dementia.[7]

As part of GENFI, participants were followed-up annually or every 2 years via a semi-structured health interview, neurological and neuropsychological examination, blood sample collection, and MRI. Knowledgeable informants (eg, spouse or sibling) were interviewed about potential changes in cognition or behaviour. Global cognitive functioning was scored using the Mini Mental State Examination (MMSE) and the Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale (FTLD-CDR); we used changes in MMSE and FTLD-CDR as measures of clinical decline.

Participants were considered symptomatic (either at baseline or during follow-up) on the basis of international consensus criteria.[1,2] Symptom onset was defined as the moment that symptoms were first noted retrospectively by caregivers. The presence of concomitant amyotrophic lateral sclerosis was defined according to revised El Escorial criteria.[11] Presymptomatic mutation carriers had no evidence of motor deficits or behavioural or cognitive changes, as assessed by neurological and neuropsychological examination and structured informant interviews.

Local ethics committees at each site approved the study and all participants provided written informed consent. Clinical researchers were masked to the genetic status of at-risk individuals unless participants had undergone predictive testing. Such participants wished to know their mutation status (ie, whether they are presymptomatic mutation carriers or non-carriers), and underwent predictive testing through a clinical geneticist.

Procedures

Blood was collected by venipuncture in serum-separating tubes and centrifuged (2000 g for 10 min) at room temperature within 3 h of withdrawal, according to a standardised GENFI protocol. After centrifugation, serum was stored at –80°C until use. Participants were not instructed to fast and time of day at blood collection was variable.

Serum NfL was measured in duplicate in longitudinal serum samples collected between June 8, 2012, and Dec 8, 2017, using the Simoa NF-Light Advantage Kit (Quanterix; Billerica, MA, USA) on a Simoa HD-1 Analyzer instrument, according to the manufacturer's instructions. Samples with a coefficient of variation of greater than 15% were remeasured. Samples were analysed in nine runs, with longitudinal samples of each participant measured in the same run. Laboratory technicians were masked to clinical information.

T1-weighted volumetric imaging was done using a standardised GENFI exam card[10] on three Tesla MRI scanners. All MRI scans were visually checked for artefacts before image processing, according to a standardised GENFI protocol. Each MRI scan was coupled with a serum sample with a maximum interval of 6 months between the serum sample and scan. Follow-up imaging was done on the same scanner as the baseline visit.

T1-weighted MRI scans were divided into cortical and subcortical regions, as previously described,[10] using an atlas propagation and label fusion strategy,[12] combining regions of interest to calculate grey matter cortical volumes (frontal, temporal, parietal, occipital, cingulate and insular cortices), subcortical volumes (hippocampus, amygdala, caudate nucleus, putamen, and thalamus), and cerebellar volume of both hemispheres combined. We measured whole-brain grey matter volumes using a semi-automated segmentation method.[13] Total intracranial volume was measured with SPM12 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK) as the combination of grey matter, white matter, and CSF segmentations.[14] To ensure accurate delineation of regional volumes, segmentation output files were visually checked by experts at a central neuroimaging facility. All grey matter volumes were expressed as a percentage of total intracranial volume.

Outcomes

The primary outcome was the course of NfL over time across the various stages of genetic frontotemporal dementia, and was centrally assessed. Secondary outcomes were differences in NfL concentrations between groups at baseline; diagnostic accuracy of NfL concentration at baseline; associations at baseline between NfL concentrations and imaging and clinical parameters; longitudinally, the association between change in NfL concentration and change in imaging and clinical parameters; and longitudinally, the timing of NfL increases in participants who converted to the symtomatic stage during follow-up.

Statistical analysis

We did no formal sample size or power calculations as analyses were done retrospectively. The follow-up duration for each individual was defined as the time between the first and the last available serum sample (data cutoff Dec 8, 2017).

For cross-sectional analyses, we identified three groups: symptomatic mutation carriers, presymptomatic mutation carriers (including those who converted to the symptomatic stage during follow-up), and non-carriers. We compared groups using Kruskal-Wallis tests with post-hoc Dunn's test, because NfL was not normally distributed. NfL was normally distributed after log-transformation, as confirmed by Kolmogorov-Smirnov tests and visual inspection of histogram and Q-Q plots (appendix p 4). We compared log(NfL) between clinical groups by ANCOVA, adjusting for age; disease duration was included as a covariate in comparisons between symptomatic mutation carriers. NfL was correlated with each of the regional brain volumes (model A1), MMSE (model A2), and FTLD-CDR (model A3) through multiple linear regression, adjusting for age, sex, and study site and, in brain volume analyses, for MRI scanner type. MMSE and FTLD-CDR analyses were restricted to symptomatic mutation carriers to study whether the severity of cognitive and functional deficits during the course of frontotemporal dementia was correlated with NfL. Diagnostic performance of NfL was assessed via the area under the curve (AUC) obtained by receiver operating characteristic analyses, with optimal cutoffs determined by the highest Youden's index.

We fitted a linear regression model to analyse whether baseline NfL in presymptomatic mutation carriers

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See **Online** for appendix

	Symptomatic carriers	Presymptomatic carriers	Non-carriers	p value
n	59	149	127	NA
Sex, n (%)				
Men	36 (61%)	52 (35%)	58 (46%)	0·002*
Women	23 (39%)	97 (65%)	69 (54%)	..
Age, years	63 (58–69)	45 (39–55)	50 (39–59)	<0·0001†
MMSE	25 (8–30)	30 (24–30)	30 (25–30)	<0·0001‡
FTLD-CDR	4·8 (2·5–9·5)	0 (0)	0 (0)	<0·0001‡
Serum NfL, pg/ml	52 (24–69)	9 (6–13)	8 (6–11)	<0·0001§
Follow-up duration, years (range)	1·2 (0·5–8·1)	2·1 (0·7–5·6)	2·2 (0·8–4·9)	NA
Samples per participant (range)	2 (2–5)	3 (2–6)	2 (2–6)	NA

Continuous variables are median (IQR). Phenotypes of symptomatic mutation carriers: behavioral variant FTD (n=40), primary progressive aphasia (n=11), FTD with amyotrophic lateral sclerosis (n=5), FTD with progressive supranuclear palsy (n=1), memory-predominant FTD (n=1), FTD not otherwise specified (n=1). FTD=Frontotemporal dementia. NfL= neurofilament light chain. MMSE=Mini Mental State Examination. FTLD-CDR=Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale. NA=not applicable. *Pearson χ^2 test. †Symptomatic carriers were older than presymptomatic carriers and non-carriers, both overall and for each genotype separately (all comparisons p<0·0001, Kruskal-Wallis test with post-hoc Dunn's test). ‡Symptomatic carriers had lower MMSE and higher FTLD-CDR than presymptomatic carriers and non-carriers (both comparisons p<0·0001, Kruskal-Wallis tests with post-hoc Dunn's test). §Symptomatic carriers had higher NfL concentrations than presymptomatic carriers and non-carriers (both comparisons p<0·0001, ANCOVA on log-transformed NfL levels with correction for age).

Table 1: **Baseline characteristics**

	Symptomatic carriers			Presymptomatic carriers			p value
	GRN	*C9orf72*	*MAPT*	GRN	*C9orf72*	*MAPT*	
n	25	24	10	79	46	24	NA
Age, years	61 (56–67)	68 (62–74)	58 (56–63)	48 (39–57)	43 (38–55)	39 (33–45)	<0·0001*
Age at symptom onset, years	58 (54–63)	63 (55–69)	55 (52–57)	NA	NA	NA	0·039 †
Disease duration at baseline, years	2·6 (1·1–4·6)	4·0 (2·0–6·6)	2·8 (1·3–7·4)	NA	NA	NA	0·144‡

Continuous variables are median (IQR). NA=not applicable. *Symptomatic carriers were older than presymptomatic carriers and non-carriers, both overall and for each genotype separately (all comparisons p<0·0001, Kruskal-Wallis test with post-hoc Dunn's test). †Symptomatic *C9orf72* mutation carriers were older at symptom onset than *MAPT* mutation carriers (p=0·033, Kruskal-Wallis test with post-hoc Dunn's test). ‡Kruskal-Wallis test.

Table 2: **Gene-specific baseline characteristics**

differed compared with non-carriers as they approached their expected disease onset (model B). The large variation in onset age within families would render analyses based on family onset age invalid.[10,15,16] Therefore, we used age as a proxy to approaching symptom onset. We used log-transformed baseline NfL and included age, mutation status (mutation carrier or non-carrier), and an interaction between these terms. Polynomials or natural cubic splines of age did not improve the model fit. In the case of a significant interaction term, estimated NfL at ages 40–60 years with 2-year intervals was compared between mutation carriers and non-carriers, with Bonferroni correction.

For longitudinal analyses, we identified four groups: symptomatic mutation carriers, presymptomatic mutation carriers (who remained presymptomatic during follow-up), converters (who developed frontotemporal dementia during follow-up), and non-carriers. We analysed NfL changes using linear mixed-effects models to account for the correlation between repeated measurements in each participant. We specified the following fixed-effects: time (time=0 at first serum sample), clinical group (non-carrier, presymptomatic carrier, converter, symptomatic carrier, and non-carriers as the reference group), age, sex, study site, and an interaction term between time and clinical group (model C). We included random intercepts and slopes of time per participant. NfL was log-transformed to meet the models' assumptions. We selected appropriate random and fixed effects structures using likelihood ratio tests. Polynomials or natural cubic splines of time did not improve the model fit. Differences in NfL change over time between mutation groups were studied through post-hoc analyses with an interaction term between time and the combination of mutation group and clinical status.

We calculated rates of NfL change using mixed-effects models with time as the fixed effect and a random slope and intercept of time per participant (model D). We correlated grey matter volume with rate of NfL change by mixed-effects models with age, sex, study site, and MRI scanner type as covariates, and an interaction between time and rate of NfL change to study whether rate of NfL change was associated with grey matter volume change over time (model E1). We correlated NfL change with MMSE and FTLD-CDR change in symptomatic carriers using the same approach (model E2 and E3). Formulas for all statistical models are shown in the appendix (p 2). We did all statistical analyses in *R* and SPSS (version 24). Statistical significance was set at 0·05 (two-sided).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for he decision to submit for publication.

Results

We enrolled 335 participants from 14 centres collaborating in GENFI. The final dataset included 59 symptomatic (25 with mutations in *GRN*, 24 *C9orf72*, and ten *MAPT*) and 149 presymptomatic (79 with mutations in *GRN*, 46 *C9orf72*, and 24 *MAPT*) mutation carriers, and 127 non-carriers (tables 1, 2). We included 2–6 serum samples for each participant from distinct time points, totalling 891 samples. A small subset of these samples was collected before participants had been included in GENFI. The median follow-up duration between the first and last sample was 2·1 years (appendix pp 3, 10). Nine presymptomatic mutation carriers (six for *GRN*, one for *C9orf72*, and two for *MAPT*) converted to the symptomatic stage during follow-up. Five symptomatic *C9orf72* mutation carriers had concomitant amyotrophic lateral sclerosis.

The mean coefficient of variation of duplicate NfL measurements was 4·7% (range 0–15%). The mean between-run coefficient of variation of quality control samples was 8·3% (range 3·7–12%). Four samples were excluded owing to visual haemolysis and one sample was excluded owing to a coefficient of variation of greater than 15% and insufficient serum to rerun the measurement. The number of participants remained unchanged as additional follow-up samples were available for each of these participants. T1-weighted volumetric imaging was available at baseline in 276 participants and at follow-up in 258 participants (2–4 scans per participant, minimum interval between scans: 6 months).

Baseline NfL in symptomatic mutation carriers (median 52 pg/mL [IQR 24–69]) was higher than in presymptomatic carriers (9 pg/mL [6–13]) and non-carriers (8 pg/mL [6–11]; both p<0·0001). These differences were also seen for each mutation group separately. Symptomatic *GRN* mutation carriers had higher baseline NfL (69 pg/mL) than did symptomatic *C9orf72* mutation carriers (39 pg/mL; p=0·005; after exclusion of patients with frontotemporal dementia and amyotrophic lateral sclerosis: 37 pg/mL; p=0·004) and *MAPT* mutation carriers (20 pg/mL; p<0·0001). Correction for age and, in the latter comparison, disease duration on log-transformed NfL yielded similar p values (figure 1A).

Age correlated with NfL (r_s 0·770; p<0·0001). This correlation was also present when restricted to non-carriers (r_s 0·754; p<0·0001), with an estimated increase of 1·2% per year. No difference in NfL was found between men and women (9·6 pg/mL *vs* 9·2 pg/mL; p=0·101).

Overall, baseline NfL did not differ significantly between presymptomatic mutation carriers and non-carriers (p=0·892). However, modelled by age (model B), an interaction was seen between mutation status and age (p=0·045), with higher NfL in presymptomatic mutation carriers than in non-carriers from the age of 48 years (contrast estimate at 48 years 0·065 [SE 0·024]; p=0·033; figure 1B).

Receiver operating characteristic analyses of baseline NfL showed a high AUC to separate symptomatic from presymptomatic mutation carriers (AUC 0·93 [95% CI 0·90–0·97]); sensitivity 86%, specificity 87% at cutoff concentration 17 pg/mL) and to separate symptomatic mutation carriers from non-carriers (AUC 0·95 [95% CI 0·92–0·98]; sensitivity 88%, specificity 91% at cutoff concentration 15 pg/mL; appendix p 5).

Baseline NfL was correlated with baseline grey matter volume of the whole brain (p=0·0003), frontal lobe (p<0·0001), insula (p=0·0006), cingulate gyrus (p=0·005), and temporal lobe (p=0·045; appendix p 12).

In symptomatic mutation carriers, baseline NfL was correlated with baseline MMSE (n=55; p=0·003), but not with baseline FTLD-CDR (n=48; p=0·221; appendix p 12). No association was found between NfL and disease duration (n=59; *b*=0·003 [SE 0·009]; p=0·749).

The mixed-effects model (model C) of NfL change over time showed an overall interaction between time and clinical status on NfL (F-test=10·6; p<0·0001; appendix p 14). Non-carriers had relatively stable NfL concentrations over time during follow-up (figure 2A; appendix p 6). Two non-carriers had high NfL at baseline with large decreases during follow-up.

Across all non-converting presymptomatic mutation carriers, we found an increase in log(NfL) over time (*b*=0·015 [SE 0·007]; p=0·044; appendix p 14; figure 2A). Post-hoc analyses showed an increase in presymptomatic *C9orf72*-(*b*=0·030 [SE 0·011]; p=0·005), but not in presymptomatic *GRN* (p=0·528) or *MAPT* (p=0·298) mutation carriers (appendix p 15). We visually identified

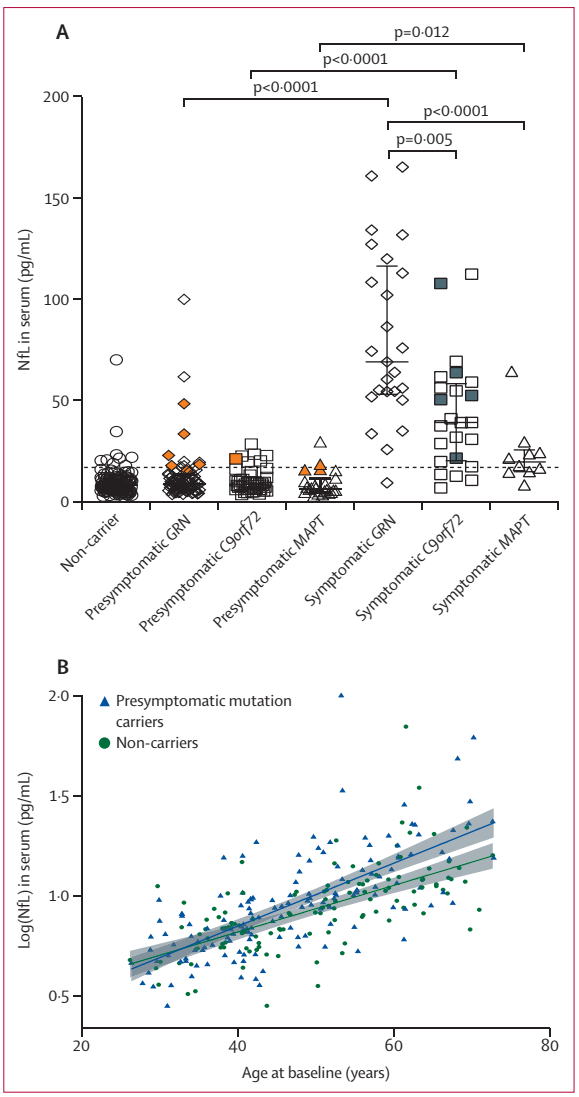

Figure 1: **Neurofilament light chain concentration at baseline**
(A) Baseline NfL in presymptomatic and symptomatic mutation carriers and non-carriers. Participants in orange were presymptomatic at baseline and converted to the symptomatic stage during follow-up. Grey squares show symptomatic *C9orf72* mutation carriers with both frontotemporal dementia and amyotrophic lateral sclerosis. The dashed horizontal line is the suggested cutoff value of 17 pg/mL to separate symptomatic mutation carriers from presymptomatic mutation carriers. Reported p values are from ANCOVA on log-transformed NfL concentrations with correction for age and, in comparisons between symptomatic mutation carriers, disease duration. Error bars represent median and IQR. (B) Baseline log(NfL) in presymptomatic mutation carriers and non-carriers. Curves were drawn by a linear regression model with an interaction term for age by carrier status (p=0·045; model B). Shaded areas represent 95% CIs. For masking purposes, the displayed x-axis range is 25–80 years (nine participants not shown) and a jitter of ± 2 years was added to all participants (analyses were done on raw data). NfL=neurofilament light chain.

seven (non-converting) presymptomatic mutation carriers (four for *C9orf72* and three for *GRN*; median age at baseline 63 years) with large increases during follow-up (appendix p 7).

An increase in NfL over time was seen in converters (*b*=0·097 [SE 0·018]; p<0·0001; figures 3, 2A). The group

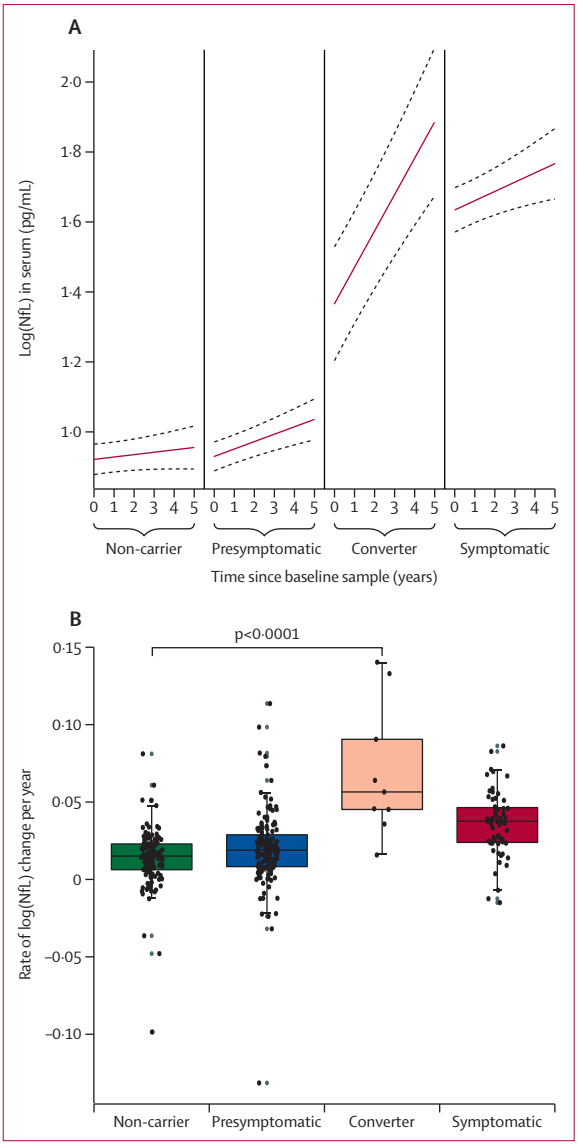

Figure 2: **Neurofilament light chain change over time**
(A) Estimated NfL trajectories for each clinical group, with curves drawn using mixed-effects modelling (model C), for participants of median age. Dashed lines are 95% CIs. (B) Rates of NfL change per year across clinical groups. The boxes map to the median, 25th and 75th quartiles, and whiskers extend to 1·5 × IQR. NfL=neurofilament light chain.

of nine converters had higher NfL at baseline (before symptom onset) than did non-converting presymptomatic mutation carriers (median 19 pg/mL [IQR 17–28] *vs* 8 pg/mL [6–11]; p=0·0007; corrected for age). Receiver operating characteristic analyses showed an AUC of 0·93 (95% CI 0·89–0·98) to distinguish converters from presymptomatic mutation carriers using baseline NfL (sensitivity 100%, specificity 84%, cutoff 15·0 pg/mL; appendix p 5). Details for each converter are shown in the appendix (pp 8, 11).

In symptomatic mutation carriers, NfL did not change during follow-up (*b*=0·017 [SE 0·010]; p=0·101) and

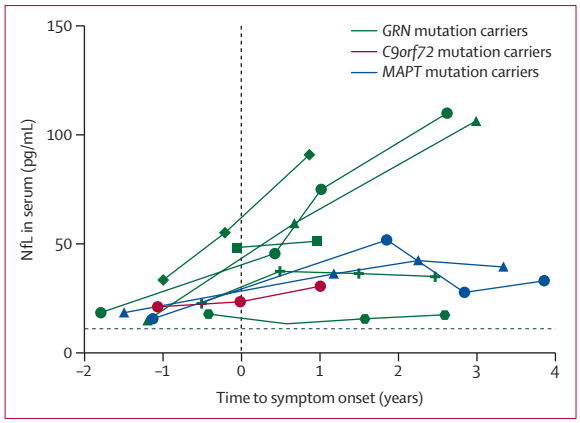

Figure 3: **Individual neurofilament light chain trajectories in converters**
The dashed horizontal line marks the baseline median NfL concentration in non-converting presymptomatic mutation carriers. NfL=neurofilament light chain.

remained elevated (figure 2A). Post-hoc analyses showed an increase over time in *GRN* mutation carriers (*b*=0·040 [SE 0·017]; p=0·019), with much variation in individual NfL trajectories (appendix pp 7), whereas, in symptomatic *C9orf72* and *MAPT* mutation carriers, NfL remained stable over time (p=0·650 for *C9orf72* and p=0·464 for *MAPT*; appendix p 15).

The rate of NfL change was higher in converters than in non-carriers and non-converting presymptomatic mutation carriers (both p<0·0001; model D; figure 2B).

Across all groups, the rate of NfL change was associated with the change over time in volume of the frontal lobe, insula, cingulate gyrus, hippocampus, putamen (all p<0·0001), whole brain volume (p=0·001), temporal lobe (p=0·001), amygdala (p=0·012) and cerebellum (p=0·026) (figure 4; coefficients shown in the appendix p 16). The rate of NfL change was associated with MMSE change over time (n=49; *b*=–94·7 [SE 33·9]; p=0·003; appendix p 9), but not with FTLD-CDR change (n=47; *b*=–3·46 [SE 46·3]; p=0·941; models E1–E3). Results for all statistical models are given in the appendix (pp 12–16).

Discussion

This longitudinal study of the largest cohort of presymptomatic and symptomatic genetic frontotemporal dementia showed stable NfL concentrations in most presymptomatic mutation carriers, a significant NfL increase over conversion to the symptomatic stage, and stable, elevated NfL over the course of frontotemporal dementia. Increases in NfL were associated with more pronounced atrophy rates in several brain regions.

We found elevated blood NfL in participants with genetic frontotemporal dementia, with good diagnostic accuracy to distinguish symptomatic from presymptomatic mutation carriers, in accordance with previous cross-sectional studies.[4–6] The correlation between cross-sectional NfL and MMSE supports the clinical relevance of this biomarker. We confirmed the previous finding of especially high NfL in *GRN*-associated frontotemporal

dementia,[4,5,17,18] which could be due to extensive white matter pathology.[19]

We describe three major findings regarding presymptomatic NfL increases. First, in converters, baseline NfL (1–2 years before symptom onset) was higher than in non-converting presymptomatic mutation carriers. Similar findings have been reported in familial amyotrophic lateral sclerosis.[20] Second, we found higher baseline NfL in presymptomatic mutation carriers than in non-carriers from the age of 48 years. These presymptomatic NfL increases probably reflect early axonal damage in a prodromal disease stage,[21] which might be a promising intervention time for disease-modifying therapies. The good diagnostic accuracy of baseline NfL to distinguish converters from non-converting carriers (albeit in small numbers) highlights the potential value of serum NfL as a candidate selection tool. More pre-conversion data are needed to determine whether the rate of NfL change might be even more sensitive to imminent conversion. Most converters in the present study were *GRN* mutation carriers, which might—to some extent—have driven the overall NfL increase in converters. In future studies, it will be interesting to study gene-specific differences in the timing of NfL increases. Finally, the large NfL increases seen in a small number of non-converting presymptomatic mutation carriers raise the question of whether these participants are approaching conversion. Further follow-up evaluations as part of GENFI will reveal whether this is the case.

The stable NfL concentrations in *C9orf72* and *MAPT* symptomatic carriers in our study are consistent with previous findings in sporadic behavioural variant frontotemporal dementia,[18] amyotrophic lateral sclerosis,[20,22,23] and familial Alzheimer's disease.[24] However, in *GRN* mutation carriers, an overall increase over time was seen with substantial fluctuations in NfL trajectories. Such fluctuations could hamper the use of NfL as a biomarker of treatment effect. Further research is needed to elucidate confounding factors of NfL in *GRN* mutation carriers. One possible explanation could lie in the severity of neuroinflammation, which is thought to have an important role in *GRN*-associated frontotemporal dementia.[25] Correlative analyses with longitudinal inflammatory biomarkers could be insightful for this purpose.

The correlation between rate of NfL change and atrophy rate of several brain regions is similar to previously reported associations for grey matter atrophy in primary progressive aphasia and familial and sporadic Alzheimer's disease.[24,26,27] This finding suggests that the speed of neuronal breakdown might determine the amount of NfL shed into the extracellular fluid and, ultimately, into the blood. The association of NfL with cerebellar volume could be driven by the *C9orf72* mutation carriers, for whom prominent cerebellar atrophy has been described.[28] The prominent associations with subcortical structures support the hypothesis that areas rich in large-calibre myelinated axons contribute more strongly to NfL release,

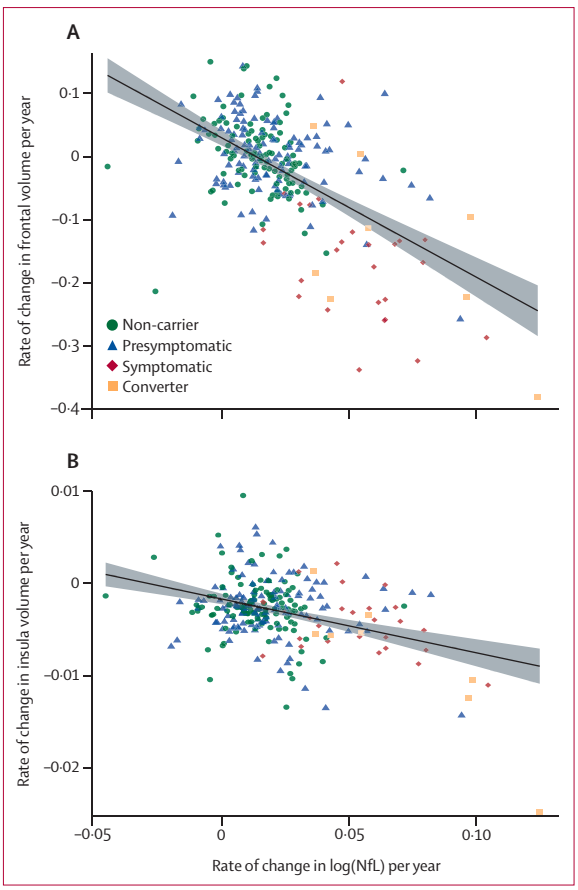

Figure 4: **Association between annual rate of log(NfL) change and rate of brain volume change**
Data are frontal volume change (A) and insular volume change (B), as extracted from linear mixed-effects models (model D) in non-carriers, presymptomatic carriers, symptomatic carriers, and converters. Shaded areas are 95% CIs. All brain volumes are expressed as a percentage of total intracranial volume.

as NfL is an axonal protein.[7,18] It will be interesting to know whether NfL changes correlate with longitudinal white matter measures, such as diffusion tensor imaging.

The absence of a correlation between changes in NfL and changes in FTLD-CDR is not entirely surprising, since most symptomatic mutation carriers had stable NfL despite functional deterioration. NfL changes might have preceded major functional decline; more sensitive measures of early symptoms, such as neuropsychological test scores or behavioural measures, could be more suitable for these analyses.[10]

Why NfL increases around conversion, and appears to stabilise in most symptomatic mutation carriers, is unexplained. The release and accumulation of NfL is presumably counterbalanced by clearing mechanisms.[22] The presence of autoantibodies against NfL, as described in patients with amyotrophic lateral sclerosis,[29,30] could contribute to this equilibrium. The observed NfL increases and decreases in some symptomatic mutation carriers could be explained by disturbances in this equilibrium (eg, during periods of more rapid or slow

brain atrophy). Notably, NfL decreases have also been described in some patients with behavioural variant frontotemporal dementia[18] and primary progressive aphasia.[26]

In two non-carriers and two presymptomatic mutation carriers, we found high NfL at baseline with rapid decreases over the follow-up period. We found no evidence of sample processing or assay-based causes for these unexpected fluctuations. Although brief medical history and neurological examination did not reveal any relevant neurological disorders, asymptomatic or minor (transient) neurological comorbidities cannot be ruled out as causative factors. A more detailed understanding of confounding factors of serum NfL is important for its clinical application and requires further study.

Major strengths of this study are the large number of presymptomatic and symptomatic mutation carriers, all of whom had multiple NfL measurements, and the availability of corresponding neuroimaging datasets. The inclusion of carriers of pathogenic mutations allowed us to investigate pathologically homogeneous cohorts, in contrast to studies of patients with clinically defined frontotemporal dementia. Accurate measurement of NfL was ensured using ultrasensitive Simoa technology, which offers superior analytical sensitivity compared with ELISA and electrochemiluminescence.[7,31] Finally, we included samples from mutation carriers across the entire spectrum of disease, from presymptomatic to advanced stages of frontotemporal dementia.

A weakness of this study is that reporting of symptom onset was based, as in a clinical setting, on retrospective estimations given by a caregiver, which could introduce a certain amount of inaccuracy owing to the insidious nature of frontotemporal dementia. Inevitably, in converters, a certain time interval exists between symptom onset and the diagnosis of frontotemporal dementia. We ensured that this interval did not influence our estimates of NfL increase by plotting individual NfL changes against symptom onset rather than diagnosis. The risk of bias due to the use of data from multiple centres was probably diminished through the use of standardised protocols and statistical correction for study sites. The association between the slope of NfL change and changes over time in brain volume and clinical parameters must be interpreted considering limitations of the applied statistical model, which used the estimated NfL slope as a fixed effect and, therefore, did not account for variability of this estimation. Finally, for correlative neuroimaging analyses, we used combined volumes for left and right hemispheres and did not account for asymmetric atrophy.

In summary, our findings highlight the value of serum NfL as an easily accessible biomarker in genetic frontotemporal dementia. Repeated measurements might be a suitable measure of disease activity in mutation carriers before symptom onset. Replication of our findings in a larger dataset with longer follow-up, allowing for longitudinal evaluation of NfL with more complex statistical models, is needed to confirm this hypothesis. The characterisation of NfL over the course of genetic frontotemporal dementia provides valuable information for its use as a surrogate marker of treatment effect in therapeutic trials.

Contributors

ELvdE and JCvS contributed to data acquisition, conception and design of the study, statistical analysis, and drafting of the manuscript and figures. JDR and LHM contributed to data acquisition and conception and design of the study. DR contributed to the statistical analyses. IMWV and CET performed NfL measurements. The remaining authors recruited patients and collected data. All authors critically reviewed the manuscript and approved the final draft.

Declaration of interests

We declare no competing interests.

Acknowledgments

We thank all participants and their family members for taking part in this study. This study was supported in the Netherlands by two Memorabel grants from Deltaplan Dementie (The Netherlands Organisation for Health Research and Development and Alzheimer Nederland; grant numbers 733050813 and 733050103), the Bluefield Project to Cure Frontotemporal Dementia, the Dioraphte foundation (grant number 1402 1300), and the European Joint Programme – Neurodegenerative Disease Research and the Netherlands Organisation for Health Research and Development (PreFrontALS: 733051042, RiMod-FTD: 733051024); in Spain by the EU Joint Programme – Neurodegenerative Disease Research (JPND) network PreFrontALS (01ED1512/AC14/00013) and Fundacio Marato de TV3 (grant number 20143810); in Sweden by grants from JPND Prefrontals Swedish Research Council (VR) 529-2014-7504, Swedish Research Council (VR) 2015-02926, Swedish Research Council (VR) 2018-02754, Swedish Frontotemporal Dementia Initiative- Schörling Foundation, Swedish Brain Foundation, Swedish Alzheimer Foundation, Stockholm County Council ALF, Karolinska Institutet Doctoral Funding and StratNeuro, Swedish Demensfonden, during the conduct of the study; in Italy by the Italian Ministry of Health (Ricerca Corrente); in Germany by the JPND network PreFrontAls (01ED1512), the German Federal Ministry of Education and Research (FTLDc O1GI1007A), the EU (FAIR-PARK II), the German Research Foundation/DFG (VO2028, SFB1279), the foundation of the state Baden-Württemberg (D.3830) and the Thierry Latran Foundation; in the United Kingdom by the Medical Research Council UK (MR/M023664/1), Alzheimer Society (AS-PG-15-025) and Alzheimer's Research UK. JDR is an MRC Clinical Scientist (MR/M008525/1) and has received funding from the NIHR Rare Diseases Translational Research Collaboration (BRC149/NS/MH). JL reports consulting fees from Aesku, speaker's fees from Bayer Vital, speaker's fees from Willi Gross Foundation, consulting fees from Axon Neuroscience, consulting fees from Ionis Pharmaceuticals, and non-financial support from Abbvie, all outside the submitted work. JBR is supported by the Wellcome Trust (103838) and the National Institute for Health Research Cambridge Biomedical Research Centre, and Holt Fellowship.

References

1 Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 2011; **134:** 2456–77.

2 Gorno-Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. *Neurology* 2011; **76:** 1006–14.

3 Lashley T, Rohrer JD, Mead S, et al. Review: an update on clinical, genetic and pathological aspects of frontotemporal lobar degenerations. *Neuropathol Appl Neurobiol* 2015; **41:** 858–81.

4 Meeter LH, Dopper EG, Jiskoot LC, et al. Neurofilament light chain: a biomarker for genetic frontotemporal dementia. *Ann Clin Transl Neurol* 2016; **3:** 623–36.

5 Rohrer JD, Woollacott IO, Dick KM, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. *Neurology* 2016; **87:** 1329–36.

6 Scherling CS, Hall T, Berisha F, et al. Cerebrospinal fluid neurofilament concentration reflects disease severity in frontotemporal degeneration. *Ann Neurol* 2014; **75:** 116–26.

7 Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol* 2018; **14:** 577–89.

8 Varhaug KN, Torkildsen O, Myhr KM, et al. Neurofilament light chain as a biomarker in multiple sclerosis. *Front Neurol* 2019; **10:** 338.

9 Bacioglu M, Maia LF, Preische O, et al. Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. *Neuron* 2016; **91:** 56–66.

10 Rohrer JD, Nicholas JM, Cash DM, et al. Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis. *Lancet Neurol* 2015; **14:** 253–62.

11 Brooks BR, Miller RG, Swash M, et al. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000; **1:** 293–99.

12 Cardoso MJ, Modat M, Wolz R, et al. Geodesic information flows: spatially-variant graphs and their application to segmentation and fusion. *IEEE Trans Med Imaging* 2015; **34:** 1976–88.

13 Freeborough PA, Fox NC, Kitney RI. Interactive algorithms for the segmentation and quantitation of 3-D MRI brain scans. *Comput Methods Programs Biomed* 1997; **53:** 15–25.

14 Malone IB, Leung KK, Clegg S, et al. Accurate automatic estimation of total intracranial volume: a nuisance variable with less nuisance. *Neuroimage* 2015; **104:** 366–72.

15 Seelaar H, Kamphorst W, Rosso SM, et al. Distinct genetic forms of frontotemporal dementia. *Neurology* 2008; **71:** 1220–26.

16 van Swieten JC, Heutink P. Mutations in progranulin (GRN) within the spectrum of clinical and pathological phenotypes of frontotemporal dementia. *Lancet Neurol* 2008; **7:** 965–74.

17 Goossens J, Bjerke M, Van Mossevelde S, et al. Diagnostic value of cerebrospinal fluid tau, neurofilament, and progranulin in definite frontotemporal lobar degeneration. *Alzheimers Res Ther* 2018; **10:** 31.

18 Steinacker P, Anderl-Straub S, Diehl-Schmid J, et al. Serum neurofilament light chain in behavioral variant frontotemporal dementia. *Neurology* 2018; **91:** e1390–401.

19 Sudre CH, Bocchetta M, Cash D, et al. White matter hyperintensities are seen only in GRN mutation carriers in the GENFI cohort. *Neuroimage Clin* 2017; **15:** 171–80.

20 Benatar M, Wuu J, Andersen PM, et al. Neurofilament light: a candidate biomarker of presymptomatic amyotrophic lateral sclerosis and phenoconversion. *Ann Neurol* 2018; **84:** 130–39.

21 Weston PSJ, Poole T, O'Connor A, et al. Longitudinal measurement of serum neurofilament light in presymptomatic familial Alzheimer's disease. *Alzheimers Res Ther* 2019; **11:** 19.

22 Lu CH, Macdonald-Wallis C, Gray E, et al. Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis. *Neurology* 2015; **84:** 2247–57.

23 Verde F, Steinacker P, Weishaupt JH, et al. Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2019; **90:** 157–64.

24 Preische O, Schultz SA, Apel A, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med* 2019; **25:** 277–83.

25 Chitramuthu BP, Bennett HPJ, Bateman A. Progranulin: a new avenue towards the understanding and treatment of neurodegenerative disease. *Brain* 2017; **140:** 3081–104.

26 Steinacker P, Semler E, Anderl-Straub S, et al. Neurofilament as a blood marker for diagnosis and monitoring of primary progressive aphasias. *Neurology* 2017; **88:** 961–69.

27 Mattsson N, Cullen NC, Andreasson U, et al. Association Between longitudinal plasma neurofilament light and neurodegeneration in patients with Alzheimer disease. *JAMA Neurol* 2019; **76:** 791–99.

28 Meeter LH, Kaat LD, Rohrer JD, et al. Imaging and fluid biomarkers in frontotemporal dementia. *Nat Rev Neurol* 2017; **13:** 406–19.

29 Fialova L, Svarcova J, Bartos A, et al. Cerebrospinal fluid and serum antibodies against neurofilaments in patients with amyotrophic lateral sclerosis. *Eur J Neurol* 2010; **17:** 562–66.

30 Puentes F, Topping J, Kuhle J, et al. Immune reactivity to neurofilament proteins in the clinical staging of amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2014; **85:** 274–78.

31 Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med* 2016; **54:** 1655–61.