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Abstract: Background: Frontotemporal dementia (FTD) is frequently caused by genetic mutations in GRN, C9orf72 and MAPT. Neurofilament light chain (NfL) is a promising blood biomarker in genetic FTD, with elevated levels in symptomatic mutation carriers. A better understanding of NfL dynamics is essential for its use in upcoming therapeutic trials. We investigated longitudinal serum NfL trajectories in presymptomatic and symptomatic genetic FTD.

Methods: In this multicenter cohort study, we measured NfL by Simoa technology in 2-6 longitudinal serum samples of 59 symptomatic and 149 presymptomatic carriers of a mutation in GRN, C9orf72 or MAPT, and 127 non-carriers participating in the Genetic FTD Initiative (GENFI). Nine presymptomatic carriers became symptomatic during follow-up ('converters'). Using mixed effects models, we analysed NfL changes over time and correlated them with longitudinal imaging and clinical parameters.
Findings: Baseline NfL was strongly elevated in symptomatic carriers (median (interquartile range) 52 pg/ml (24-69)) compared to presymptomatic carriers (9 pg/ml (6-13);$p<0\cdot001$) and non-carriers (8 pg/ml (6-11);$p<0\cdot001$). Baseline NfL was higher in converters than in non-converting carriers (19 pg/ml (17-28) vs. 8 pg/ml (6-11);$p<0\cdot001$). During follow-up, NfL remained stable in most presymptomatic carriers, and increased in converters ($p<0.001$). In symptomatic C9orf72 and MAPT mutation carriers, NfL was stable, while in symptomatic GRN mutation carriers a further increase was seen ($p=0\cdot015$). The rate of NfL change

over time was associated with atrophy rate in several grey matter regions, but not with rate of change in clinical parameters.

Interpretation: This study confirms the value of blood NfL as a disease progression biomarker in genetic FTD and indicates that longitudinal NfL measurements could help identify mutation carriers approaching symptom onset and capture the rate of brain atrophy. The stable levels in C9orf72- and MAPT-associated FTD offer potential for NfL as a marker of treatment effect in therapeutic trials.

Funding: "Memorabel" (ZonMw), Bluefield Project

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LONGITUDINAL SERUM NEUROFILAMENT LIGHT CHAIN IN GENETIC FRONTOTEMPORAL DEMENTIA

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ABSTRACT

Background: Frontotemporal dementia (FTD) is frequently caused by genetic mutations in *GRN*, *C9orf72* and *MAPT*. Neurofilament light chain (NfL) is a promising blood biomarker in genetic FTD, with elevated levels in symptomatic mutation carriers. A better understanding of NfL dynamics is essential for its use in upcoming therapeutic trials. We investigated longitudinal serum NfL trajectories in presymptomatic and symptomatic genetic FTD.

Methods: In this multicenter cohort study, we measured NfL by Simoa technology in 2-6 longitudinal serum samples of 59 symptomatic and 149 presymptomatic carriers of a mutation in *GRN*, *C9orf72* or *MAPT*, and 127 non-carriers participating in the Genetic FTD Initiative (GENFI). Nine presymptomatic carriers became symptomatic during follow-up ('converters'). Using mixed effects models, we analysed NfL changes over time and correlated them with longitudinal imaging and clinical parameters.

Findings: Baseline NfL was strongly elevated in symptomatic carriers (median (interquartile range) 52 pg/ml (24-69)) compared to presymptomatic carriers (9 pg/ml (6-13);p<0·001) and non-carriers (8 pg/ml (6-11);p<0·001). Baseline NfL was higher in converters than in non-converting carriers (19 pg/ml (17-28) vs. 8 pg/ml (6-11);p<0·001). During follow-up, NfL remained stable in most presymptomatic carriers, and increased in converters (p<0.001). In symptomatic *C9orf72* and *MAPT* mutation carriers, NfL was stable, while in symptomatic *GRN* mutation carriers a further increase was seen (p=0·015). The rate of NfL change over time was associated with atrophy rate in several grey matter regions, but not with rate of change in clinical parameters.

Interpretation: This study confirms the value of blood NfL as a disease progression biomarker in genetic FTD and indicates that longitudinal NfL measurements could help identify mutation carriers approaching symptom onset and capture the rate of brain atrophy. The stable levels in *C9orf72*- and *MAPT*-associated FTD offer potential for NfL as a marker of treatment effect in therapeutic trials.

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1. INTRODUCTION

Frontotemporal dementia (FTD) is a common cause of young onset dementia and is characterised by progressive behavioural and/or language changes.[1,2] Autosomal dominant inheritance is present in 20-30% of cases, most commonly due 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 moment to start treatment, likely in the preclinical stage, and as a surrogate endpoint to measure treatment effect.

Cross-sectional studies have shown that neurofilament light chain (NfL), a constituent of the axonal cytoskeleton, is a promising diagnostic and prognostic blood biomarker in genetic FTD, with low levels in presymptomatic mutation carriers and high levels in symptomatic mutation carriers.[4-6] NfL is elevated in various other neurological diseases, likely reflecting neuro-axonal degeneration.[7] In multiple sclerosis, NfL decreases have been observed after anti-inflammatory treatment,[8] and in Alzheimer's disease (AD) mouse models, decreases were seen following inhibition of amyloid-β lesions,[9] suggesting that NfL is a dynamic marker of disease activity.

Thus far, it is unknown when NfL starts to increase and how NfL changes over the course of FTD. The Genetic FTD Initiative (GENFI), which follows carriers of mutations in *GRN, C9orf72* and *MAPT*, provides an opportunity to prospectively study disease progression from presymptomatic to overt FTD and to identify biomarkers of early pathologic processes.

In this study, we longitudinally measured serum NfL using an ultrasensitive Single Molecule Array (Simoa) in the GENFI cohort to evaluate its temporal profile in genetic FTD. We used corresponding brain imaging and clinical datasets to study whether NfL changes correlate with rates of brain atrophy and clinical decline.

2. METHODS

2.1 Subjects

335 subjects were included from 14 centres collaborating in GENFI, which follows patients with FTD due to a pathogenic mutation in *GRN, MAPT* or *C9orf72* (symptomatic mutation carriers) and healthy at-risk first-degree relatives (either presymptomatic mutation carriers or non-carriers).[10] Participants

were included in the current study if at least two serum samples were available with a time interval of six months or more. Exclusion criteria included neurological comorbidities likely to affect NfL levels, including cerebrovascular events.[7] The final dataset included 59 symptomatic (25 *GRN*, 24 *C9orf72*, 10 *MAPT*) and 149 presymptomatic (79 *GRN,* 46 *C9orf72,* 24 *MAPT*) mutation carriers and 127 non-carriers. We included 2-6 serum samples for each subject from distinct time points, with a total of 891 samples. The median follow-up duration between the first and last sample was 2·1 years.

As part of GENFI, participants were followed yearly or two-yearly by a semi-structured health interview, neurological and neuropsychological examination, blood sample collection and MR imaging. Knowledgeable informants (e.g. spouse, sibling) were interviewed about potential changes in cognition and/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).

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

2.2 Standard protocol approvals and patient consents

Local ethics committees at each site approved the study and all participants provided written informed consent. Clinical researchers were blinded to genetic status of at-risk individuals if they had not undergone predictive testing.

2.3 Sample collection, processing and storage

Blood samples were collected by venipuncture in SST-tubes and centrifuged at 2000g for 10 minutes at room temperature within three hours of withdrawal according to a standardised GENFI protocol. After centrifugation, serum was stored in aliquots at -80°C until use. Participants were not instructed to fast and time of day at blood collection was variable.

2.4 Laboratory methods

Serum NfL was measured in duplicate using the Simoa NF-Light Advantage Kit from Quanterix on a

Simoa HD-1 Analyzer instrument according to the manufacturer's instructions. The mean coefficient of variation (CV) of duplicate measurements was 4·7% (range 0-15%); samples with a CV>15% were re-measured. Samples were analysed in nine independent runs; longitudinal samples of each subject were measured in the same run. The mean between-run CV of quality control samples was 8·3% (range 3·7-12%). Four samples were excluded due to visual hemolysis, and one sample was excluded due to a CV>15% and insufficient serum to rerun the measurement. The number of subjects remained unchanged as additional follow-up samples were available for each of these subjects. Laboratory technicians were blinded to clinical information and mutation status.

2.5 Neuroimaging analyses

T1-weighted volumetric imaging was available at baseline in 276 subjects and at follow-up in 258 subjects (2-4 scans per subject, minimum interval between scans: six months). Follow-up imaging was acquired on the same scanner as the baseline visit. All MRI scans were acquired using a standardised GENFI exam card[10] on 3 Tesla MRI scanners and were visually checked for artefacts prior to image processing according to a standardised GENFI protocol. Each MRI scan was coupled to a serum sample with a maximum interval of six months between the serum sample and scan.

T1-weighted MRI scans were parcellated 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 (separated into frontal, temporal, parietal, occipital, cingulate and insular cortices), subcortical volumes (hippocampus, amygdala, caudate nucleus, putamen, thalamus) and cerebellar volume of both hemispheres combined (http://www.neuromorphometrics.org:808/seg/). We measured whole-brain grey matter volumes using a semi-automated segmentation method.[13] Total intracranial volume (TIV) 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, all segmentation output files were visually checked by an expert.

For cross-sectional analyses, grey matter volumes were expressed as a percentage of TIV, whereas for longitudinal analyses of within-subject change, raw volumes were used.

2.6 Statistical analyses

Statistical analyses were performed in *R* and IBM SPSS Statistics 24. Statistical significance was set at 0.05 (two-sided).

2.6.1 Cross-sectional analyses of baseline data

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. Group comparisons were performed using Kruskal-Wallis tests with post-hoc Dunn's test, as NfL was not normally distributed. Additionally, we compared (normally distributed) log-transformed baseline NfL between clinical groups adjusting for age by ANCOVA; disease duration was included as a covariate in comparisons between symptomatic mutation carriers. Baseline NfL was correlated with clinical measures and each of the regional brain volumes using Spearman's partial rank correlation, adjusting for age, sex and study site and, in brain volume analyses, for MRI scanner type. Bonferroni correction for multiple testing was applied where appropriate. Diagnostic performance of serum NfL was assessed by areas under the curve (AUC) obtained by receiver operating characteristic (ROC) analyses, with optimal cut-off levels determined by the highest Youden's index.

We fit a linear regression model to analyse whether baseline NfL in presymptomatic mutation carriers differed compared to non-carriers as they approached their expected disease onset (model A). All subjects who were asymptomatic at baseline were included. The previously reported 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 baseline age, mutation status (mutation carrier or non-carrier) and an interaction between these terms. Adding non-linear terms did not improve the model fit. In the case of a significant interaction term, estimated NfL levels at ages 40-60 with 2-year intervals were compared between mutation carriers and non-carriers, with Bonferroni correction for multiple testing.

2.6.2 Longitudinal analyses of follow-up data

For longitudinal analyses we identified four groups: symptomatic mutation carriers, presymptomatic mutation carriers (who remained presymptomatic during follow-up), converters (who developed FTD 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 subject. These models are robust to unbalanced data. We specified the following fixed effects: time (time = 0 at first serum sample), clinical group (non-carrier, presymptomatic carrier, converter, symptomatic carrier, with non-carriers as the reference group), age, sex, study site and interaction terms between time and clinical group (model B). In subgroup analyses which compared NfL changes among symptomatic *GRN, C9orf72* and *MAPT* mutation carriers, disease duration was also included as a fixed effect. We included random intercepts and slopes of time per subject. NfL was log-transformed to meet the models' assumptions. Appropriate random and fixed effects structures were selected using likelihood ratio tests. Adding a non-linear effect of time did not improve the model fit.

Next, we calculated individual rates of change in NfL, each of the regional brain volumes, MMSE and FTLD-CDR (all log-transformed) by extracting their slopes using simplified mixed effects models, with time as the fixed effect and a random slope and intercept of time per subject (model C). NfL slopes were correlated with regional brain volume slopes by linear regression with age, sex, study site and MRI scanner type as covariates (model D). We correlated NfL slopes with slopes of MMSE and FTLD-CDR in a similar way, but limited the analyses to symptomatic mutation carriers (model E), as the inclusion of non-carriers and presymptomatic mutation carriers would severely skew the distribution of test scores, thereby violating normality assumptions.

Finally, using linear regression, we compared rates of change in NfL between subjects who were presymptomatic at baseline to non-carriers at various ages, analogous to the analyses described for baseline NfL (model F).

2.7 Role of the funding source

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

3. RESULTS

3.1 Subjects

Subject characteristics at baseline are shown in table 1. Nine presymptomatic mutation carriers (6

GRN, 1 *C9orf72,* 2 *MAPT*) converted to the symptomatic stage during follow-up. Five symptomatic *C9orf72* mutation carriers had concomitant symptoms of ALS.

3.2 Baseline NfL

Baseline NfL levels in symptomatic mutation carriers (median 52 pg/ml) were significantly higher than in presymptomatic mutation carriers (9 pg/ml) and non-carriers (8 pg/ml; both $p<0{\cdot}001$). These differences were also seen for each mutation group separately. Symptomatic *GRN* mutation carriers had higher baseline NfL levels (69 pg/ml) than symptomatic *C9orf72 (*39 pg/ml;$p=0{\cdot}005$; after exclusion of FTD-ALS cases: 37 pg/ml;$p=0{\cdot}004$) and *MAPT* mutation carriers (20 pg/ml;$p<0{\cdot}001$). Correction for age and, in the latter comparison, disease duration, on log-transformed NfL yielded similar p-values (figure 1).

Age correlated significantly with NfL levels ($r_s=0{\cdot}770$;$p<0{\cdot}001$). This correlation was similarly present when limited to non-carriers ($r_s=0{\cdot}754$;$p<0{\cdot}001$), with an estimated increase of 1·2% per year.

Overall, baseline NfL did not differ significantly between presymptomatic mutation carriers and non-carriers (figure 1). However, when modelled by age (model A), significantly higher NfL levels were found in presymptomatic mutation carriers compared to non-carriers from the age of 48 years (contrast estimate=0·065;standard error (SE)=0·024;$p=0{\cdot}033$; interaction term between mutation status and age: $p=0{\cdot}040$).

ROC 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 of 86% and specificity of 87% at a cut-off level of 17 pg/ml) and to separate symptomatic mutation carriers from non-carriers (AUC 0·95 [95% CI 0·92-0·98]) with a sensitivity of 88% and specificity 91% at a cut-off level of 15 pg/ml).

Across all groups, baseline NfL was correlated with frontal lobe grey matter volume ($r_s=-0{\cdot}208$;$p=0{\cdot}007$) and insular volume ($r_s=-0{\cdot}192$;$p=0{\cdot}019$).

Baseline NfL was correlated with MMSE (n=317; $r_s=-0{\cdot}254$; $p<0{\cdot}001$) and with FTLD-CDR (n=144; $r_s=0{\cdot}519$; $p<0{\cdot}001$). When limited to symptomatic mutation carriers, a significant correlation was found with MMSE (n=51;$r_s=-0{\cdot}382$;$p=0{\cdot}005$) but not with FTLD-CDR or disease duration.

3.3 Longitudinal NfL

Non-carriers had relatively stable NfL levels over time during follow-up. Two non-carriers had high NfL levels at baseline with large decreases during follow-up (figures 1, 2).

Across all non-converting presymptomatic mutation carriers, a small but significant increase of NfL over time was found (β=0·015;SE=0·007;p=0·043;model B). We visually identified seven (non-converting) presymptomatic mutation carriers (4 *C9orf72,* 3 *GRN*, median age at baseline 63 years) with large NfL increases over follow-up (figure 3).

The group of nine converters showed significantly higher NfL levels at baseline (before symptom onset) than non-converting presymptomatic mutation carriers (median 19 versus 8 pg/ml, corrected for age by ANCOVA, p=0·001). A significant increase in NfL over time was seen in converters (β=0·097;SE=0·018;p<0·001;model B) (figure 4). NfL levels and clinical characteristics of each converter are shown in supplementary table 1.

In symptomatic mutation carriers, NfL levels did not change during follow-up (β=0·016;SE=0·011;p=0·149;model B) and remained elevated. When split into genetic subtypes, a significant increase over time was found in *GRN* mutation carriers (β=0·042;SE=0·017;p=0·015), with much variation in individual NfL trajectories (figure 3). In symptomatic *C9orf72* and *MAPT* mutation carriers, NfL did not change over time (p=0·816 and p=0·518 respectively).

The estimated NfL trajectories for each clinical group are shown in figure 5.

3.3.1 Rates of NfL change

The rate of NfL change, as extracted from model C, was significantly higher in converters than in non-carriers and non-converting presymptomatic mutation carriers (both p<0·001) (figure 6A). When modelled by age (model F), a higher rate of change was seen in presymptomatic mutation carriers compared to non-carriers from the age of 46 years (contrast estimate=0·069;SE=0·015;p=0·041; interaction between age and carrier status: p=0·039) (figure 6B). A borderline significant correlation was found between rate of NfL change and disease duration among converters and symptomatic mutation carriers (r_s=-0·237;p=0·051;n=68).

3.3.2 Correlations with longitudinal neuroimaging and clinical parameters

Across all groups, the rate of NfL change over time was significantly negatively associated with the slope of cingulate gyrus (β=-6·266;SE=1·174), insula (β=-4·221;SE=0·538), frontal cortex (β=-4·785;SE=0·634;p<0·001), temporal cortex (β=-4·822;SE=0·974), putamen (β=-3·375; SE=0·370) (all p<0·001) and caudate nucleus (β=-1·727;SE=0·539;p=0·020) (figure 7) (model C, D).

These results remained significant when limiting the analyses to symptomatic mutation carriers, except for the temporal cortex which did not withstand multiple testing correction.

We found no correlation between the rate of NfL change over time and the slope of FTLD-CDR in symptomatic mutation carriers (n=47;p=0·511) or MMSE (n=49;p=0·342) (model C, E).

4. DISCUSSION

The present longitudinal study of the largest cohort of (pre)symptomatic genetic FTD showed stable NfL levels in most presymptomatic mutation carriers, a significant NfL increase over conversion to the symptomatic stage, and stable, elevated NfL levels over the course of FTD. Increases in NfL were associated with a more pronounced rate of atrophy in several brain regions.

We found markedly elevated blood NfL in patients with genetic FTD, 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 FTLD-CDR and MMSE supports the clinical relevance of this biomarker. We confirmed the previous finding of especially high NfL in *GRN*-associated FTD,[4,5,17,18] which may be due to extensive white matter pathology.[19]

We describe three major findings with regards to presymptomatic NfL increases. First, in converters, baseline NfL levels (1-2 years before symptom onset) were higher than in non-converting presymptomatic mutation carriers. Similar findings have been reported in familial ALS.[20] Second, we found higher baseline NfL levels in presymptomatic mutation carriers compared to non-carriers from the age of 48 years, with an even earlier divergence in rate of NfL change. These presymptomatic NfL increases likely reflect early axonal damage in a prodromal disease stage,[21] which may be a promising intervention-time for disease-modifying therapies. If confirmed in a larger number of converters, serum NfL could potentially be used as a candidate selection tool. Most converters in the present study were

GRN mutation carriers, which may to some extent have driven the overall NfL increase in converters. In future studies it will be interesting to look at possible gene-specific differences in the timing of NfL increase. Finally, the large NfL increases observed in a small number of non-converting presymptomatic mutation carriers, raise the question whether perhaps these subjects are approaching conversion. Additional follow-up visits as part of the GENFI study will reveal whether or not this is the case.

The stable NfL levels in *C9orf72* and *MAPT* symptomatic carriers in the present study are consistent with observations in series of sporadic behavioural variant FTD,[18] ALS[20,22,23] and familial AD.[24] In *GRN* mutation carriers, on the other hand, an overall increase over time was seen with substantial fluctuations in NfL trajectories. Such fluctuations could hamper the potential use of NfL as a biomarker of treatment effect in individuals. Further research is needed to elucidate confounding factors of NfL levels in *GRN* mutation carriers. One possible explanation may lie in the severity of neuro-inflammation, which is thought to play an important role in *GRN*-associated FTD.[25] Correlative analyses of longitudinal inflammatory biomarkers with NfL levels could be insightful for this purpose.

The correlation between the 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 (PPA) and familial and sporadic AD.[24,26,27] It suggests that the speed of neuronal breakdown may determine the amount of NfL shed into the extracellular fluid and ultimately into the blood. The prominent associations with subcortical structures support the hypothesis that areas rich in large-caliber myelinated axons contribute more strongly to NfL release, as NfL is an axonal protein.[7,18] It will be interesting to investigate whether NfL changes correlate with longitudinal white matter measures such as diffusion tensor imaging.

The lack of correlation between changes in NfL and rate of clinical decline (as measured by the MMSE and FTLD-CDR) indicates that these do not occur simultaneously, and is not entirely surprising since most symptomatic mutation carriers had stable NfL levels despite clinical deterioration. Possibly, NfL changes preceded changes in these clinical parameters. Perhaps more sensitive measures of early symptoms, such as neuropsychological test scores or behavioural measures, would be more suitable for these analyses.[10]

Thus far unexplained is why NfL increases around conversion, and appear to stabilise in most symptomatic mutation carriers. The release and accumulation of NfL is presumably counterbalanced by clearing mechanisms.[22] The presence of auto-antibodies against NfL, as previously described in ALS-patients, could contribute to this equilibrium.[28,29] The observed NfL increases and decreases in some symptomatic mutation carriers could be explained by disturbances in this equilibrium, for example during periods of more rapid or slow brain atrophy. Interestingly, NfL decreases were also previously described in some patients with behavioural variant FTD[18] and PPA.[26]

In two non-carriers and two presymptomatic mutation carriers, we found high NfL levels at baseline with rapid decreases over follow-up. 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 as causative factors cannot be ruled out. A more detailed understanding of confounding factors of serum NfL is important for its clinical application and requires further study.

A major strength of the present study is the large number of presymptomatic and symptomatic mutation carriers, all of whom had multiple NfL measurements, and the availability of corresponding brain imaging datasets. The inclusion of carriers of pathogenic mutations allowed us to create pathologically homogeneous cohorts, in contrast to studies of patients with clinically defined FTD. Accurate measurement of NfL was ensured using the ultrasensitive Simoa technology, which offers superior analytical sensitivity compared to ELISA and electrochemiluminescence.[7,30] Finally, we included samples from mutation carriers across the entire spectrum of disease, from presymptomatic to advanced stages of FTD.

A potential weakness is that symptom onset was based, as in a clinical setting, on retrospective estimations given by a caregiver, which could introduce a certain amount of inaccuracy due to the insidious nature of FTD. Inevitably, in converters a certain time interval exists between symptom onset and the diagnosis of FTD. We ensured that this interval did not influence our estimates of NfL increase by plotting individual NfL changes against time of symptom onset rather than diagnosis. For correlative neuroimaging analyses, we used combined volumes for left and right hemispheres and therefore did not account for asymmetric atrophy; including separate left- and right-sided volumes may have led to even stronger associations. Finally, the use of data from multiple centers could introduce a bias, although this was likely diminished through the use of standardised protocols and statistical correction for study site.

In conclusion, our results underline the value of serum NfL as an easily accessible biomarker in genetic FTD. The repeated measurement of NfL may be a suitable measure of disease activity in mutation carriers prior to the onset of symptoms. Replication of our findings in an independent dataset is needed to confirm this. The individually stable NfL levels in symptomatic *C9orf72* and *MAPT* mutation carriers offer potential for NfL as a surrogate marker of treatment effect in therapeutic trials.

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AUTHOR CONTRIBUTIONS

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. IV and CET performed NfL measurements. The remaining authors recruited patients and collected data and contributed to the writing of the manuscript. All authors critically reviewed the manuscript and approved the final draft.

DECLARATION OF INTEREST

The authors report no potential conflicts of interest with respect to this manuscript.

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FIGURE AND TABLE LEGENDS

Figure 1. Baseline NfL in presymptomatic and symptomatic mutation carriers and non-carriers. Subjects in red were presymptomatic at baseline and converted to the symptomatic stage during follow-up. Blue triangles indicate symptomatic *C9orf72* mutation carriers with both FTD and amyotrophic lateral sclerosis. Dashed horizontal line indicates the suggested cut-off value of 17 pg/ml to separate symptomatic mutation carriers from presymptomatic mutation carriers. Reported p-values are from ANCOVA on log-transformed NfL levels with correction for age. **p<0·01;***p<0·001.

Figure 2. Individual NfL trajectories in non-carriers. To facilitate interpretation of visually unstable NfL trajectories, subjects with annualised changes (calculated by subtracting the first from the last NfL measurement and dividing by total follow-up time) of > 5pg/ml are highlighted in black. The remaining subjects are shown in grey.

Figure 3. Individual NfL trajectories in presymptomatic and symptomatic mutation carriers. **(A-C)** Presymptomatic *GRN, C9orf72* and *MAPT* mutation carriers; **(D-F)** Symptomatic *GRN, C9orf72* and *MAPT* mutation carriers. To facilitate interpretation of visually unstable NfL trajectories, subjects with annualised changes (calculated by subtracting the first from the last NfL measurement and dividing by total follow-up time) of > 5pg/ml are highlighted in black. For visualisation purposes, follow-up duration was limited to six years; two symptomatic mutation carriers (1 *GRN*, 1 *C9orf72*) with longer follow-up durations (8·1 and 7·7 years) had visually stable NfL levels over time.

Figure 4. Individual NfL trajectories in converters. The dashed horizontal line represents baseline median NfL level in non-converting presymptomatic mutation carriers. *GRN* mutation carriers are shown in green, *C9orf72* mutation carriers in red and *MAPT* mutation carriers in blue.

Figure 5. Estimated NfL trajectories by clinical group, with curves drawn using mixed effects modelling (model B). Dashed lines represent 95% confidence intervals. Time indicates number of years since baseline sample.

Figure 6. Rate of change in NfL per year. **(A)** Rates of NfL change across clinical groups. The boxes map to the median, 25th and 75th quartiles and whiskers extend to 1·5 x interquartile range. **(B)** Rates of NfL change in subjects who were presymptomatic mutation carriers (blue triangles) or non-carriers (green circles) at baseline. Curves were drawn by a linear regression model with a significant interaction term for age by carrier status (p=0·039). Shaded areas represent 95% confidence intervals. For blinding purposes, the displayed x-axis range is 30-80 years (9 subjects not shown) and a jitter of ±2 years was added to all subjects (analyses were performed on raw data).

Figure 7. Relationship between the slope of log(serum NfL) and the slope of **(A)** log(frontal volume) and **(B)** log(insular volume), as extracted from linear mixed effects models in non-carriers (green, presymptomatic carriers (blue), symptomatic carriers (orange) and converters (red).

Table 1. Subject characteristics at baseline. Continuous variables are described as medians (interquartile range) unless stated otherwise. Phenotypes of symptomatic mutation carriers: behavioural 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). MMSE, Mini Mental State Examination; FTLD-CDR, Frontotemporal Lobar Degeneration – Clinical Dementia Rating scale; NA, not applicable.

PANEL: RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed up 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)". While several cross-sectional studies reported elevated NfL levels in genetic FTD, we did not identify any longitudinal NfL studies in genetic FTD. A recent large longitudinal study of familial Alzheimer's disease (AD) reported an increased rate of NfL change >15 years before symptom onset, while a smaller study of familial amyotrophic lateral sclerosis (ALS) 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 FTD mutation carriers (*GRN, C9orf72* and *MAPT*) demonstrates stable NfL levels in most presymptomatic mutation carriers, a sharp increase around conversion to the symptomatic stage, and overall stable, elevated levels during the disease course. Nine presymptomatic mutation carriers who developed FTD during follow-up had elevated NfL levels 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 confirms the value of serum NfL as an easily accessible biomarker in genetic FTD. The repeated measurement of NfL appears to be a robust measure to identify mutation carriers approaching symptom onset. The overall stable NfL levels in in the symptomatic stage offer potential for NfL as a surrogate marker of treatment effect in upcoming therapeutic trials.

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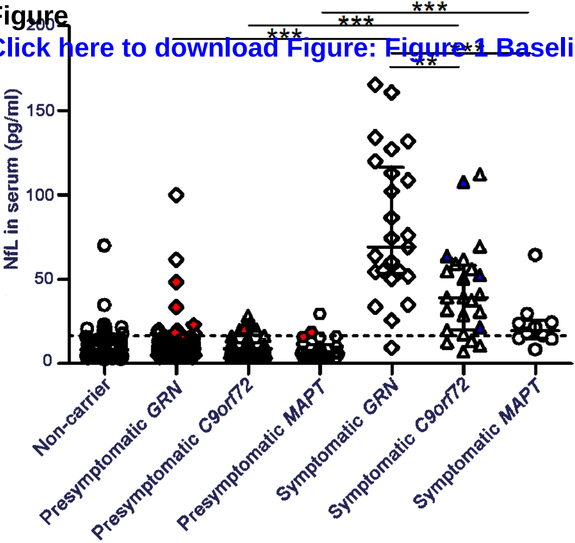

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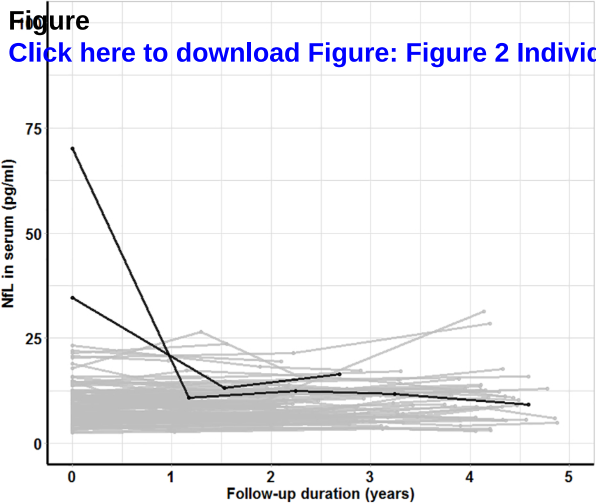

Figure

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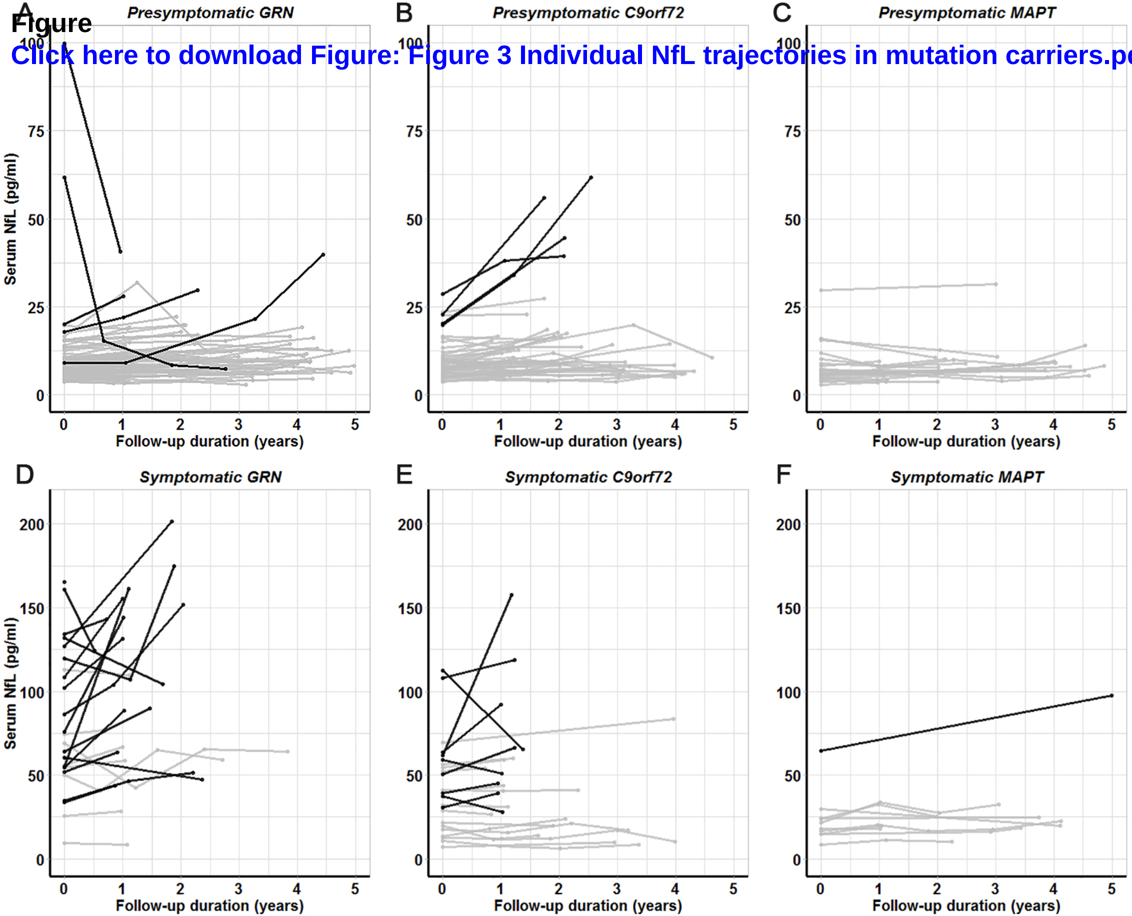

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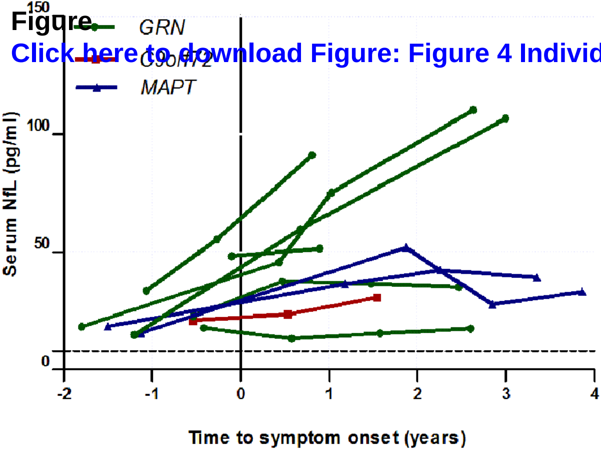

Figure

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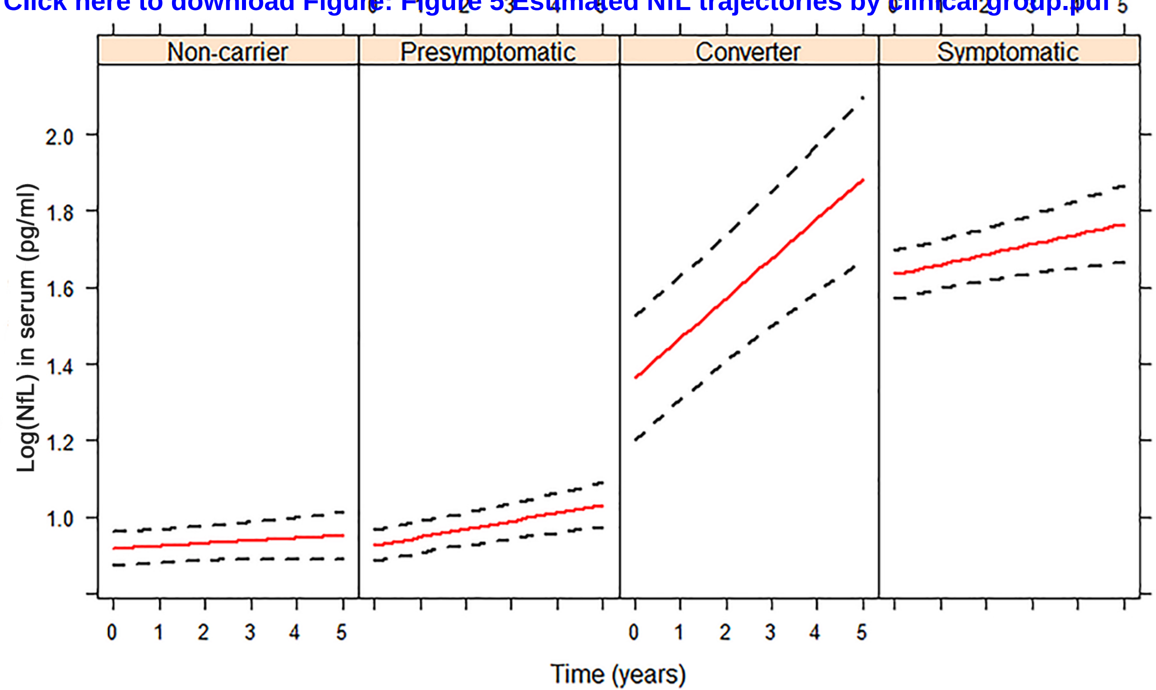

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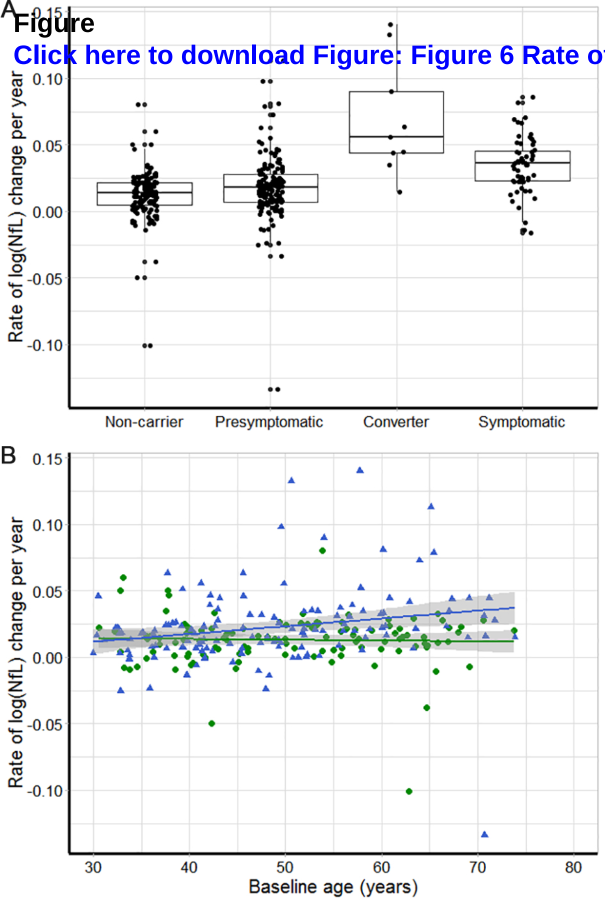

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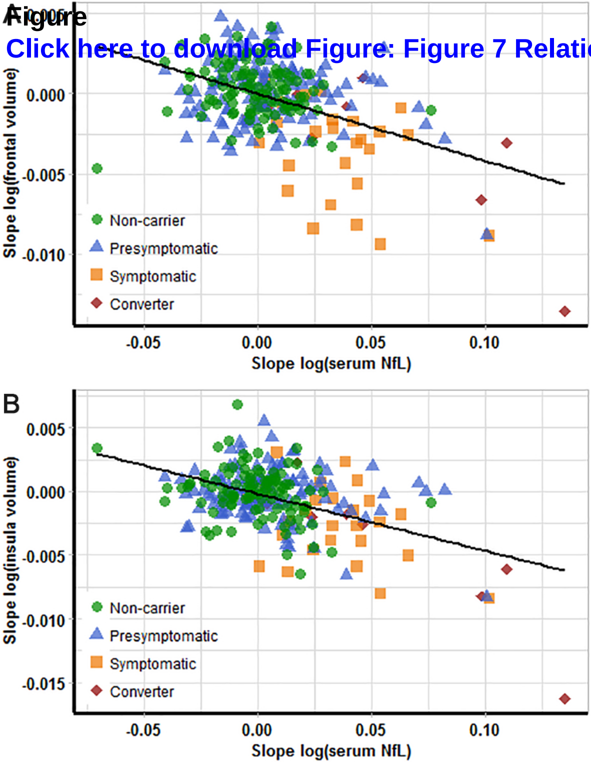

Table

Table 1. Subject characteristics at baseline. Continuous variables are described as medians (interquartile range) unless stated otherwise. 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). MMSE, Mini Mental State Examination; FTLD-CDR, Frontotemporal Lobar Degeneration – Clinical Dementia Rating scale; NA, not applicable.

	Symptomatic carriers			Presymptomatic carriers			Non-carriers	*P*
N	59			149			127	
Sex, male (%)	36 (61%)			48 (34%)			58 (46%)	0.055
Age, years	63 (58-69)			45 (39-55)			50 (39-59)	<0.001[a]
MMSE	25 (8-30)			30 (24-30)			30 (25-30)	<0.001[b]
FTLD-CDR	4.8 (2.5-9.5)			0 (0)			0 (0)	<0.001[b]
Serum NfL (pg/ml)	52 (24-69)			9 (6-13)			8 (6-11)	<0.001[c]
Follow-up duration (range)	1.2 (0.5-8.1)			2.1 (0.7-5.6)			2.2 (0.8-4.9)	NA
Samples per subject (range)	2 (2-5)			3 (2-6)			2 (2-6)	NA
Gene-specific information	*GRN*	*C9orf72*	*MAPT*	*GRN*	*C9orf72*	*MAPT*	NA	NA
N	25	24	10	79	46	24	NA	NA
Age at collection, years	61 (56-67)	68 (62-74)	58 (56-63)	48 (39-57)	43 (38-55)	39 (33-45)	NA	<0.001[a]
Age at symptom onset, years	58 (54-63)	63 (55-69)	55 (52-57)	NA	NA	NA	NA	0.039[d]
Disease duration at baseline, years	2.6	4.0	2.8	NA	NA	NA	NA	0.144

[a] Symptomatic carriers significantly older than presymptomatic carriers and non-carriers, both overall and for each genotype separately (all comparisons p<0.001).

[b] Symptomatic carriers significantly lower MMSE and higher FTLD-CDR than presymptomatic carriers and non-carriers (both comparisons p<0.001).

[c] Symptomatic carriers significantly higher NfL levels than presymptomatic carriers and non-carriers (both comparisons p<0.001).

[d] Symptomatic *C9orf72* mutation carriers significantly older at symptom onset than *MAPT* mutation carriers (p=0.033).

SUPPLEMENTARY DATA

Supplementary table 1. Clinical characteristics and NfL levels for converters. Age at symptom onset shown is as reported retrospectively by a caregiver. bvFTD, behavioural variant FTD; PPA, primary progressive aphasia; MMSE, Mini Mental State Examination; FTLD-CDR, Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale; N/A, not available.

	Visit 1	Visit 2	Visit 3	Visit 4
Converter 1: MAPT mutation carrier; male; age at symptom onset 45 years.				
NfL	18.52	36.36	42.44	39.47
Age (years)	44.0	46.7	47.8	48.9
FTLD-CDR	0	10	13.5	11.5
MMSE	30	29	27	20
Diagnosis	Presymptomatic	bvFTD		
Converter 2: MAPT mutation carrier; male; age at symptom onset 40 years.				
NfL	15.80	51.88	27.80	33.18
Age (years)	39.6	42.6	43.6	44.6
FTLD-CDR	0	0	4.5	5.5
MMSE	30	29	29	28
Diagnosis	Presymptomatic		bvFTD	
Converter 3: GRN mutation carrier; female; age at symptom onset 57 years.				
NfL	15.02	59.55	106.45	
Age (years)	56.4	58.3	60.6	
FTLD-CDR	0	1	24	
MMSE	27	26	0	
Diagnosis	Presymptomatic	Non-fluent variant PPA		
Converter 4: GRN mutation carrier; female; age at symptom onset 52 years.				
NfL	18.51	45.68	75.03	110.04
Age (years)	50.0	52.2	52.8	54.4
FTLD-CDR	0	1	2	20
MMSE	28	29	27	0
Diagnosis	Presymptomatic	Non-fluent variant PPA		
Converter 5: GRN mutation carrier; male; age at symptom onset 68 years.				
NfL	48.4	51.4		
Age (years)	67.7	68.9		
FTLD-CDR	0	8		
MMSE	28	27		
Diagnosis	Presymptomatic	PPA not otherwise specified		
Converter 6: GRN mutation carrier; female; age at symptom onset 59 years.				
NfL	17.88	13.44	15.76	17.56
Age (years)	58.5	59.5	60.5	61.5
FTLD-CDR	N/a	2	3.5	N/a
MMSE	30	30	29	N/a
Diagnosis	Presymptomatic	bvFTD		
Converter 7: C9orf72 mutation carrier; male; age at symptom onset 68 years.				
NfL	21.23	23.57	30.73	
Age (years)	67.4	68.5	69.5	
FTLD-CDR	0	N/a	10	
MMSE	28	26	26	
Diagnosis	Presymptomatic		bvFTD	
Converter 8: GRN mutation carrier; female; age at symptom onset 71 years.				
NfL	22.92	37.50	36.52	35.14
Age (years)	70.4	71.4	72.5	73.5
FTLD-CDR	N/a	N/a	4	N/a
MMSE	25	25	21	20
Diagnosis	Presymptomatic	Memory-predominant FTD		
Converter 9: GRN mutation carrier; female; age at symptom onset 55 years.				
NfL	33.52	55.13	90.94	
Age (years)	53.9	54.7	55.8	
FTLD-CDR	N/a	N/a	10.5	
MMSE	29	26	26	
Diagnosis	Presymptomatic		bvFTD	

Supplementary file 1. GENFI consortium members

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3	"multicenter cohort study"
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3	Provided in the abstract
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4	Provided in the introduction
Objectives	3	State specific objectives, including any prespecified hypotheses	4	"In this study, we longitudinally measured serum NfL using an ultrasensitive Single Molecule Array (Simoa) in the GENFI cohort to evaluate its temporal profile in genetic FTD. We used corresponding brain imaging and clinical datasets to study whether NfL changes correlate with rates of brain atrophy and clinical decline."
Methods				
Study design	4	Present key elements of study design early in the paper	4, 5	Provided in methods section 'subjects'
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4, 5	Provided in methods section 'subjects'
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4,5	Provided in the methods section 'subjects'
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed	N/A	

		Case-control study—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5	Provided in the methods section 'subjects'
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5,6	Provided in the methods sections 'Sample collection, processing and storage' and 'Laboratory methods'
Bias	9	Describe any efforts to address potential sources of bias	5	Provided in the methods section
Study size	10	Explain how the study size was arrived at	5,6	Provided in the methods sections 'Subjects', 'Laboratory methods' and 'Neuroimaging analyses'

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8	Provided in the methods section ‘Statistical analysis’
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8	Provided in the methods section ‘Statistical analysis’
		(*b*) Describe any methods used to examine subgroups and interactions	7,8	Provided in the methods section ‘Statistical analysis’
		(*c*) Explain how missing data were addressed	7,8	Provided in the methods section ‘Statistical analysis’
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	N/A	
		(*e*) Describe any sensitivity analyses	N/A	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4,6	Provided in the methods sections ‘Subjects’ and ‘Laboratory methods’
		(b) Give reasons for non-participation at each stage	N/A	
		(c) Consider use of a flow diagram	N/A	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1	
		(b) Indicate number of participants with missing data for each variable of interest	9-11	In the case of missing data, numbers of subjects included in subanalyses are provided throughout the Results section.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	5	Provided in the Methods section ‘Subjects’
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8-10	Provided throughout the Results section
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A	

		Cross-sectional study—Report numbers of outcome events or summary measures	N/A	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-11	Statistical analyses including confounders are described in the Methods section ‘Statistical anaysis’; unadjusted and adjusted results are provided in the Results section.
		(*b*) Report category boundaries when continuous variables were categorized	8-11	Provided throughout the Results section
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-11	Provided throughout the Results section
Discussion				
Key results	18	Summarise key results with reference to study objectives	11	First paragraph of Discussion section
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13	Provided in the Discussion section
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14	Final paragraph of Discussion section
Generalisability	21	Discuss the generalisability (external validity) of the study results	14	Final paragraph of Discussion section
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	8,14	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.