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Neurofilaments in Pre-Symptomatic ALS and the Impact of Genotype

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Abstract

Objective: To evaluate serum and cerebrospinal fluid (CSF) levels of phosphorylated neurofilament heavy (pNfH), and to compare these to levels of neurofilament light (NfL), as biomarkers of pre-symptomatic ALS.

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Design: The study population includes 34 controls, 79 individuals at-risk for ALS, 22 ALS patients, and 14 phenoconverters. At-risk individuals are enrolled through *Pre-Symptomatic Familial ALS* (*Pre-fALS*), a longitudinal natural history and biomarker study of individuals who are carriers of any ALS-associated gene mutation, but who demonstrate no clinical evidence of disease at the time of enrollment. pNfH and NfL in serum and CSF were quantified using established enzyme-linked immunosorbent assays.

Results: There is a longitudinal increase in serum pNfH in advance of the emergence of clinically manifest ALS. A similar pattern is observed for NfL, but with the absolute levels also frequently exceeding a normative threshold. Although CSF data are more sparse, similar patterns are observed for both neurofilaments, with absolute levels exceeding a normative threshold prior to phenoconversion. In serum, these changes are observed in the 6-12 months prior to disease among *SOD1* A4V mutation carriers, and as far back as 2 and 3.5 years respectively in individuals with a *FUS* c.521del6 mutation and a *C9ORF72* hexanucleotide repeat expansion.

Conclusions: Serum and CSF pNfH increase prior to phenoconversion. In CSF, the temporal course of these changes is similar to NfL. In serum, however, pNfH is less sensitive to pre-symptomatic disease than NfL. The duration of pre-symptomatic disease, as defined by changes in neurofilaments, may vary depending on underlying genotype.

Keywords

Amyotrophic lateral sclerosis; Neurofilaments; Biomarkers; Pre-Symptomatic; Disease Prevention

Introduction

Growing interest in the tangible possibility of early therapeutic intervention is fueling research into the pre-symptomatic phase of a range of neurodegenerative disorders. Essential to these endeavors is the development of biomarkers that either define individuals as being at heightened short-term risk of developing clinically manifest disease, or that indicate the presence of subclinical disease prior to the emergence of clinically manifest disease. The field of Alzheimer's disease (AD) research has led the way, currently using genetic risk (namely, the presenilin1 E280A mutation) as an eligibility criterion for the Alzheimer's Prevention Initiative (API) trial of crenezumab () and using positron emission tomography (PET) evidence of amyloid deposition as an eligibility criterion for the Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease (A4) Trial of solanezumab [1], with both trials enrolling cognitively normal subjects. In recent years, the concentrations of neurofilament proteins in serum and cerebrospinal fluid (CSF) have emerged as candidate biomarkers relevant to the study of a range of pre-symptomatic neurodegenerative diseases including familial AD [2–4], frontotemporal dementia [5–7], Huntington's disease [8–10], and amyotrophic lateral sclerosis [11].

Building on our recent work describing the utility of serum and CSF neurofilament light (NfL) as the earliest biochemical biomarker of pre-symptomatic ALS [11], here we present phosphorylated neurofilament heavy (pNfH) data as well as NfL results on an additional 4 phenoconverters (including 2 with a *C9orf72* repeat expansion). We also show how levels of these neurofilaments may shed light on the relationship between genotype and the duration

of the pre-symptomatic phase of ALS and yield an opportunity for initiating a disease prevention trial in ALS. The interest in exploring pNfH in addition to NfL rests on the potential insights that the combined fluid expression of these isoforms may yield in understanding the early evolution of pathology in ALS. The recently reported increase of the NfL isoform and the more modest up-regulation of heavy and medium chain subunits in blood from patients with ALS may reflect differences in the immunological clearance of these proteins [12] and/or an energy-saving change in the stoichiometry of distressed neuronal cells, whereby increased NfL production compensates for the overall loss of neurofilament proteins[13].

Methods

Study Population

Pre-Symptomatic Familial ALS (Pre-fALS) [14] is a longitudinal natural history and biomarker study of individuals recruited from across North America who are carriers of any ALS-causing gene mutation (in *SOD1, C9orf72, TARDBP, FUS, VCP*, etc.) but demonstrate, at the time of enrollment, no clinical or electromyographic evidence of disease. Asymptomatic carriers of pathogenic variants in these genes comprise the only population known to be at significantly greater risk for ALS (compared to the general population), and in whom a study of pre-symptomatic disease may realistically be considered. As described elsewhere [14], participants are followed longitudinally, with phenotypic (motor and cognitive), electrophysiological, quantitative motor and imaging assessments, along with collection of biological samples (urine, blood, and CSF). Cognition and behavior are assessed using a combination of the Edinburgh Cognitive Behavioral Assessment (ECAS) [15] and a full neuropsychological test battery that assesses domains relevant to frontotemporal spectrum dysfunction that occurs in association with ALS [16]; alternate versions of tests are used to minimized the potential for learning effects. These in-person assessments are repeated approximately every 12-24 months, with intervening remote visits. Phenoconversion is defined as the emergence of definite symptoms or signs that clearly indicate manifest disease [17]; those who phenoconvert continue to be followed after developing clinically manifest disease. We thus acquire longitudinal data prior to the appearance of symptoms, around the time that symptoms begin to emerge, and in the early stages of manifest disease. In addition, ALS patients as well as controls (who are either healthy individuals with no known family history of ALS or individuals who were found, as part of their *Pre-fALS* screening, not to harbor the genetic mutation known to cause ALS in their family) are evaluated with the same set of assessments as is used for *Pre-fALS* study visits. The serum and CSF samples included in this experiment were collected at study visits that took place between January 2008 and September 2017. This study was approved by the University of Miami Institutional Review Board, and all participants provided written informed consent. The study is registered on clinicaltrials.gov ().

Sample Collection, Processing, and Storage

For serum analysis, blood was collected in a red top BD vacutainer and allowed to clot upright at room temperature for 1-2 hours. Following centrifugation (1750g for 10 minutes at 4°C) serum was aliquoted into cryogenic sterile freestanding conical microtubes

(Nalgene, Rochester, NY or Bio Plas Inc., San Rafael CA) and quickly stored at −80°C until use. CSF (free of macroscopic hemoglobin) was collected in polypropylene tubes, centrifuged (1750g for 10 minutes at 4°C), aliquoted using a sterile pipette into pre-capped polypropylene cryogenic sterile freestanding conical microtubes, frozen within ~30 minutes of collection, and stored at −80°C until use.

Neurofilament Quantification

Quantification of pNfH was performed using a CE marked ELISA (Euroimmun AG, Lübeck, Germany), which utilizes polyclonal capture and monoclonal detection antibodies to pNfH. The assay and its analytical performance have been described in detail previously [18]. Each plate contained calibrators (0-10,000pg/mL) and quality controls; if required, samples were appropriately diluted to fall within the range of the standard curve. All samples were measured in duplicate at the same dilution. All pNfH assays were performed blind to group/disease state. For serum, inter-assay coefficients of variance are below 19% and the mean intra-assay coefficients of variance are below 10%. For CSF, inter-assay coefficients of variance are below 11% and the mean intra-assay coefficients of variance are below 10%. Serum and CSF NfL were assayed as previously described [11].

Statistical Analysis

Participant characteristics are summarized in Table 1. Neurofilament concentrations that were below the level of detection (<LOD) were excluded from the summary statistics in Table 2, but included in the analyses using an imputed value of 0.03 pg/ml for pNfH or 0.37 pg/ml for NfL, which was the lowest detectable value in our data. Due to the highly skewed distribution of neurofilament concentrations and the presence of outliers, analyses were performed using non-parametric tests or natural logarithm-transformed values where applicable. For ease of interpretation of log-transformed values, a small constant was added to neurofilament values before taking the natural logarithm, so that the lowest log-transformed value is 0 (rather than a negative value). For CSF NfL, we excluded one extreme outlying value (in an ALS affected individual) that was 5-fold higher than the next highest value. Summary statistics for numerical variables are provided in mean±SD (or, for variables with a highly skewed distribution, in median and range), and for categorical variables in frequency and percentage. For phenoconverters in the *Pre-fALS* study, date of phenoconversion is determined based on the earlier of either unequivocal symptoms reported by the participant, or subclinical signs of disease detected through detailed neuromuscular examination, EMG, or cognitive/behavioral testing that clearly indicate disease [17]. For affected individuals, on the other hand, date of onset is based on participants' recollection and self-report. While current efforts are underway to obtain more rigorously defined "normative threshold" of NfL or pNfH concentrations among pre-symptomatic individuals, given the constraints of the current data it is operationally defined here as the 95th percentile of all available data among the controls. Spearman rank correlation, Wilcoxon rank-sum test, or Kruskal-Wallis test were employed in the analysis of cross-sectional data. For the analysis of longitudinal data, linear regression and mixed model analysis (with random intercept and slope, unstructured covariance structure, and Kenward-Roger degrees of freedom) were employed, adjusting for baseline age in all models. Given the potential for <LOD neurofilament values to be highly influential (e.g. the by-person slope estimate may be

skewed for individuals whose first or last neurofilament value was <LOD), longitudinal analyses were performed with and without these values. Both sets of analyses yielded similar results; results from analyses without <LOD are reported below as the estimates were less prone to high influential data points. For the comparison of neurofilament rate of increase between participant groups, the mixed models included a time x group interaction term. The level of statistical significance was set at 0.05 (two-sided); given the discovery and exploratory nature of this study, however, the focus of this manuscript is less on statistical testing but more on characterizing the neurofilament concentrations observed. All statistical analyses were performed, and graphics produced, using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Study Population

The study population (Table 1) comprises 34 controls; 79 at-risk individuals (who have remained pre-symptomatic throughout the duration of follow-up to date); 14 converters (who have undergone phenoconversion and followed longitudinally from the pre-symptomatic to the symptomatic phase); and 22 affected individuals (i.e. patients with ALS at the time of initial assessment). While the affected group includes 15 ALS patients in whom there is no identified genetic mutation, all individuals in the at-risk group are at genetic risk for disease, largely due to an *SOD1* mutation (n=27 A4V, n=22 non-A4V) or a *C9orf72* hexanucleotide repeat expansion (n=25). These at-risk individuals have been followed for a median (range) of 3.7 (1.0-8.7) years. Most of the converters were *SOD1* (especially A4V) mutation carriers, but one carried a *FUS* mutation (c.521del6), and two a *C9orf72* repeat expansion. Apart from one of the *C9orf72* converters whose initial clinical manifestations were cognitive/behavioral, the earliest clinical features were motor in all other phenoconverters (Table e1). Clinical and pNfH data are available from all participants at time of initial sample collection (“baseline”). In addition, longitudinal data are available from a subset of 13 controls, 55 at-risk, 10 converters, and 16 affected, for a total of 393 person-visits (Table 1).

The control and at-risk groups are comparable in baseline age (mean ±SD = 48±12 and 47±11 years, respectively), with the converters slightly older (52±12 years) and the affected about 10 years older (60 ±7 years; $p<0.001$ when compared to controls or at-risk). As of the cut-off date for the pNfH samples included in this experiment, we had conducted a median of 3 (and up to 7) longitudinal visits on the N=94 participants in the longitudinal subset, with varying lengths of follow-up for the different groups (Table 1).

Baseline pNfH Concentration

Baseline serum pNfH levels (Table 2) are comparable between controls (median [range] = 23 [0.03-113] pg/ml), at-risk individuals (21 [1-213] pg/ml) and phenoconverters (13 [0.6-367] pg/ml), irrespective of genotype (Figure 1). While median serum levels are, as expected, higher in patients with ALS (85 [2.0-1156] pg/ml) as compared to controls ($p<0.001$), there is substantial overlap between these groups in their range of serum pNfH values. On the other hand, while lumbar puncture was only performed in 77 of 149 (52%)

study participants at baseline, there is much less overlap in CSF pNfH values between ALS affected and either control or at-risk groups (Table 2, Figure 1). Serum and CSF pNfH levels are poorly correlated (Spearman rank correlation, r=0.09, p=0.4).

For the investigation of potential age effect, we focused on controls (who are free of disease), but also considered the at-risk group (most of whom are likely to be as yet minimally affected by disease, if at all) given its much larger samples size. While there is no correlation between age and baseline serum pNfH concentrations in controls (r=0.11, p=0.5), there is a strong correlation between age and CSF pNfH (r=0.71, p=0.003) (Table e2). The magnitude of this age-related CSF pNfH elevation, however, is negligible in the context of disease-related elevation among ALS patients (Figure 2). Moreover, neither serum nor CSF pNfH levels significantly differed by sex (Table e3) or the underlying genotype (at-risk group only, Table e4).

Longitudinal Changes in Neurofilament Concentration

Longitudinally, serum pNfH levels are relatively stable among controls, at-risk individuals who have not yet undergone phenoconversion, and ALS patients (Figure 3); the median rate of pNfH increase in these 3 groups ranges from 0.76 to 1.83 pg/ml per year, after adjusting for baseline age. In contrast, in the majority of converters with data available from multiple time points before (or shortly after) phenoconversion, we observed an increase in serum pNfH *in advance* of the appearance of manifest disease (Figure 4A), with an age-adjusted median rate of pNfH increase of 93.6 pg/ml per year. Moreover, in mixed model analyses using log-transformed pNfH values and adjusting for baseline age, the rate of pNfH increase is significantly higher in converters compared to controls (p=0.003, group x time interaction term), at-risk individuals (p<0.0001), and ALS affected (p=0.002).

The absolute level of serum pNfH, on the other hand, exceeded the normative threshold in only one individual at about 1 year prior to phenoconversion (Figure 4A). By comparison, not only is a pre-symptomatic increase in serum NfL also observed in the majority of cases, the concentration of NfL is elevated above the normative threshold in all converters [11] (Figure 4B). CSF data are sparser. In four converters we observed an elevation in the absolute level of pNfH and NfL prior to phenoconversion; these elevations occurred, ~3.5 years, 2 years, ~9 months, and shortly before phenoconversion (Figures 4C and 4D).

Relationship Between Genotype and the Timing of Neurofilament Increase

Considering all available pNfH as well as NfL data from all 14 converters (4 with only baseline data so far, 10 with longitudinal data), we observe a relationship between genotype and the timing of initial neurofilament elevation. In both serum and CSF, the elevation is observed as far back as 6-12 months prior to phenoconversion among *SOD1* A4V mutation carriers; as far back as 2 years in the single *FUS* c.521del6 converter; and as far back as 3.5 years in the *C9orf72 HRE* motor converter.

Discussion

Neurofilaments have been investigated in the pre-symptomatic phase of a range of neurodegenerative diseases (summarized in Table 3). With the exception of *TRACK-HD*,

DIAN and our own *Pre-fALS* study, these studies have largely been cross-sectional, with longitudinal data available from only small numbers of participants, few (if any) phenoconverters, and therefore little opportunity to quantify neurofilament before and after phenoconversion. Moreover, these studies have focused almost exclusively on NfL. Here, we present data showing the longitudinal change in pNfH in a large number of pre-symptomatic individuals at genetic risk for ALS, with quantification of pNfH in serum and (to a lesser extent, CSF) both before and after the emergence of clinically manifest disease. We also present updated NfL data, with additional converters added to previously published data [11]. The critical observations are a pre-symptomatic increase in pNfH and NfL among phenoconverters. While the temporal patterns of the increases in serum and CSF NfL and pNfH are comparable, there is one important difference. Namely, in serum, we observe longitudinal increases (i.e. positive slopes) in both NfL and pNfH prior to manifest disease, but the elevation in absolute levels to beyond our *a priori* conservatively defined normative threshold is consistently observed in NfL but not pNfH. Whether pNfH measured using more sensitive assays (e.g. Simoa) might yield a different conclusion is as yet unknown; it is, however, the focus of an ongoing study. In contrast to the findings in serum, CSF data, albeit sparser, show similar patterns for both NfL and pNfH. The more striking observations from our NfL data may be driven, at least in part, by the more distinct separation between the ALS affected group and the control and at-risk groups in absolute levels of serum NfL. (In CSF, on the other hand, both NfL and pNfH show comparable separation between these groups). The observation that there is significant overlap in the level of blood pNfH between controls and ALS patients is consistent with previously published data [12,18]. The greater sensitivity of serum NfL (than serum pNfH) to detecting pre-symptomatic disease is perhaps also related to the strong correlation we observed between serum and CSF in NfL ($r=0.79$, $p<0.001$) but not pNfH. This in turn, may be a function of the greater methodological difficulty of accurately quantifying serum pNfH given the tendency for pNfH to be sequestrate in hetero-aggregate immune complexes [19]. It may also reflect the recently reported energy-saving adaptive response to neurodegeneration in ALS, in which upregulation of NfL and downregulation of neurofilament medium (NfM) and NfH leads to shift in the highly conserved stoichiometry of neurofilament isoforms [13].

Importantly, having now followed a larger number of phenoconverters with different genotypes, we acquired the following insight about the relationship between the timing of neurofilament increase, post-phenoconversion survival duration, and the underlying genotype: for *SOD1* A4V, an aggressive disease with median survival ~12 months, the pre-symptomatic neurofilament increase was apparent as far back as 6-12 months prior to phenoconversion. For *FUS* c.521del6, with a post-phenoconversion survival of 3.4 years, elevated neurofilament levels were evident at least 2 years prior to phenoconversion. For *C9orf72 HRE*, we have observed elevated NfL and pNfH as far back as ~3.5 years prior to phenoconversion; survival duration for this individual is unknown as (s)he has only recently developed clinically manifest disease. While the generalizability of our findings will require further observations in phenoconverters with an even broader array of genotypes, these data suggest the intriguing possibility that the *duration* of the pre-symptomatic phase of ALS (as defined by an increase in neurofilament levels) is proportional to that of the symptomatic phase of disease That is, the pre-symptomatic phase is longer in patients with more slowly

progressive (symptomatic) disease, and shorter in patients with more aggressive manifest disease.

By providing critical insight into the pre-symptomatic phase of ALS, these neurofilament data move us closer to one of the major long-term goals of the *Pre-fALS* study [20] – namely, the design of a disease prevention or early treatment trial. A major challenge to the design and implementation of such a trial has, until now, been our inability to predict when someone at genetic risk for ALS will develop manifest disease [21]. (Parenthetically, this is less of a problem for HD in which age and CAG repeat length may be used to predict likely age of onset [22], and in familial AD in which age of onset of parents and/or other family members may similarly be used [23].) The data presented show that a rise in serum neurofilament concentration is a prognostic biomarker predicting when manifest disease is likely to emerge. For a disease prevention trial, therefore, instead of enrolling anyone at genetic risk for ALS (without knowing when manifest disease is likely to emerge), we may now enroll the subset of this population most likely to develop manifest disease within a relatively short period of time (i.e. those in whom an increase in serum neurofilament levels is observed). Alternatively put, a pre-symptomatic increase in serum neurofilament levels may be used as a biomarker eligibility criterion for a disease prevention trial. Such an approach would not only obviate unnecessarily prolonged exposure to an experimental therapeutic (among people whom might not develop disease for many years), but would also enrich the study population for at-risk individuals most likely to meet the study endpoint of phenoconversion during a defined follow-up period. Moreover, the timing of the rise in neurofilament in relationship to phenoconversion among those with an *SOD1* mutation associated with rapidly progressive disease, suggests that this may be the population in which it is most feasible to try and delay or prevent the emergence of manifest disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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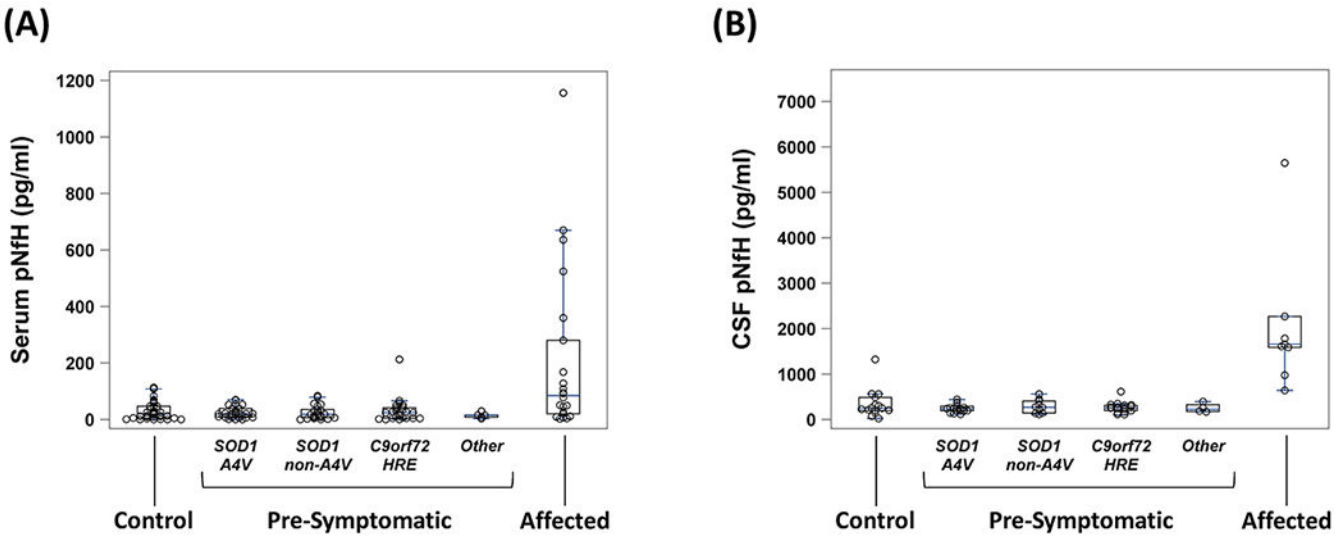

Figure 1. Baseline levels of pNfH in serum and CSF

(A) Serum pNfH (pg/ml); and (B) CSF pNfH (pg/ml). Boxes show median, and 25th and 75th percentiles; whiskers extend to a maximum of 1.5 x interquartile range (IQR), or to the most extreme value if it is less than 1.5 x IQR from the 25th or 75th percentile. CSF = cerebrospinal fluid; pNfH = phosphorylated neurofilament heavy.

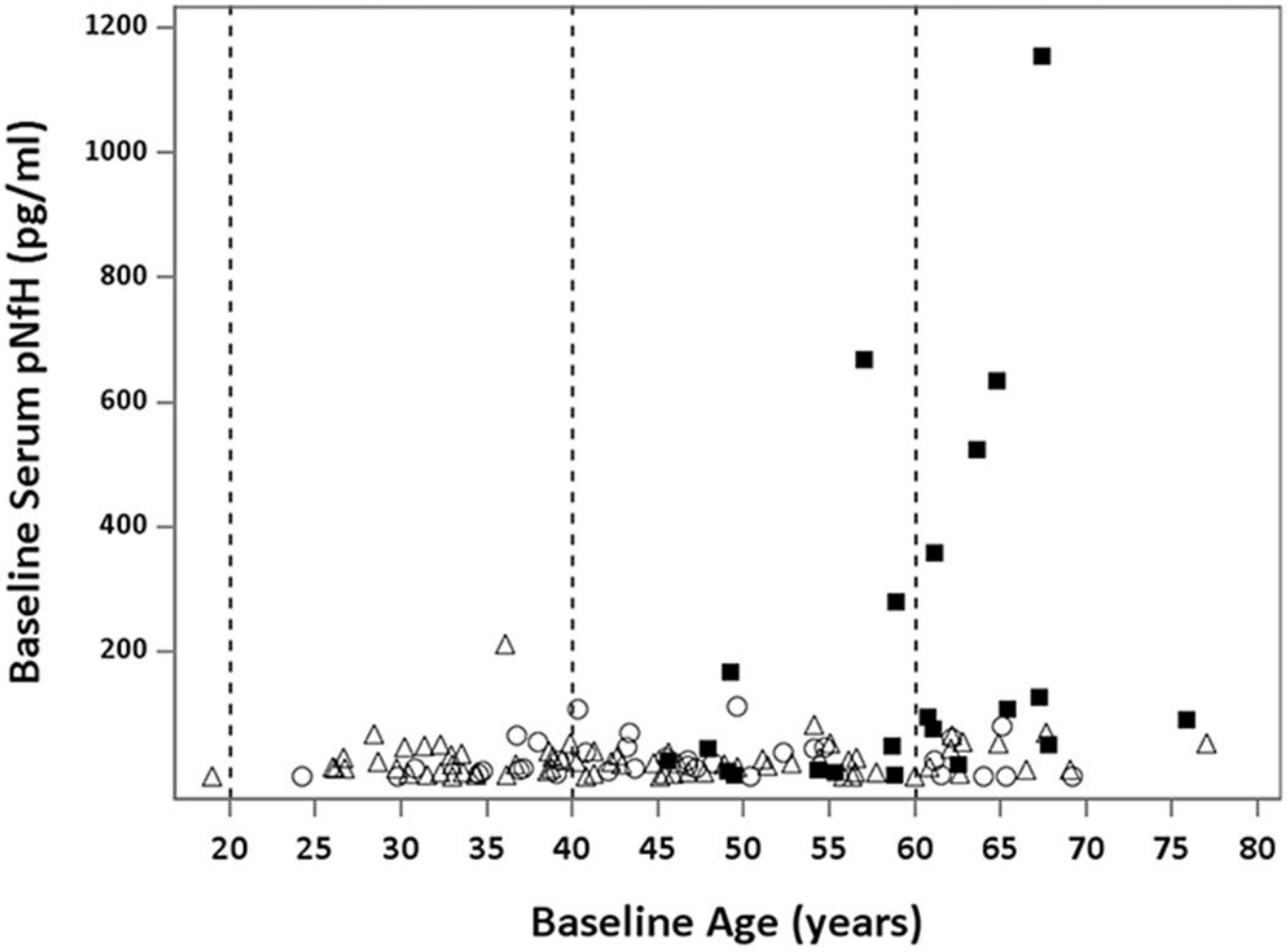

Figure 2. Scatterplot of baseline serum pNfH levels vs. age

Although pNfH levels are slightly higher among older individuals in the control group (open circles) and at-risk group (open triangles), the magnitude of this increase is negligible in comparison to the levels of pNfH observed among ALS patients (closed squares); that is, older age does not explain the increase in serum pNfH among ALS patients. Vertical dashed lines demarcate age groups (< 40, 40-60 and > 60 years). ALS = amyotrophic lateral sclerosis; NfL = neurofilament light; pNfH = phosphorylated neurofilament heavy.

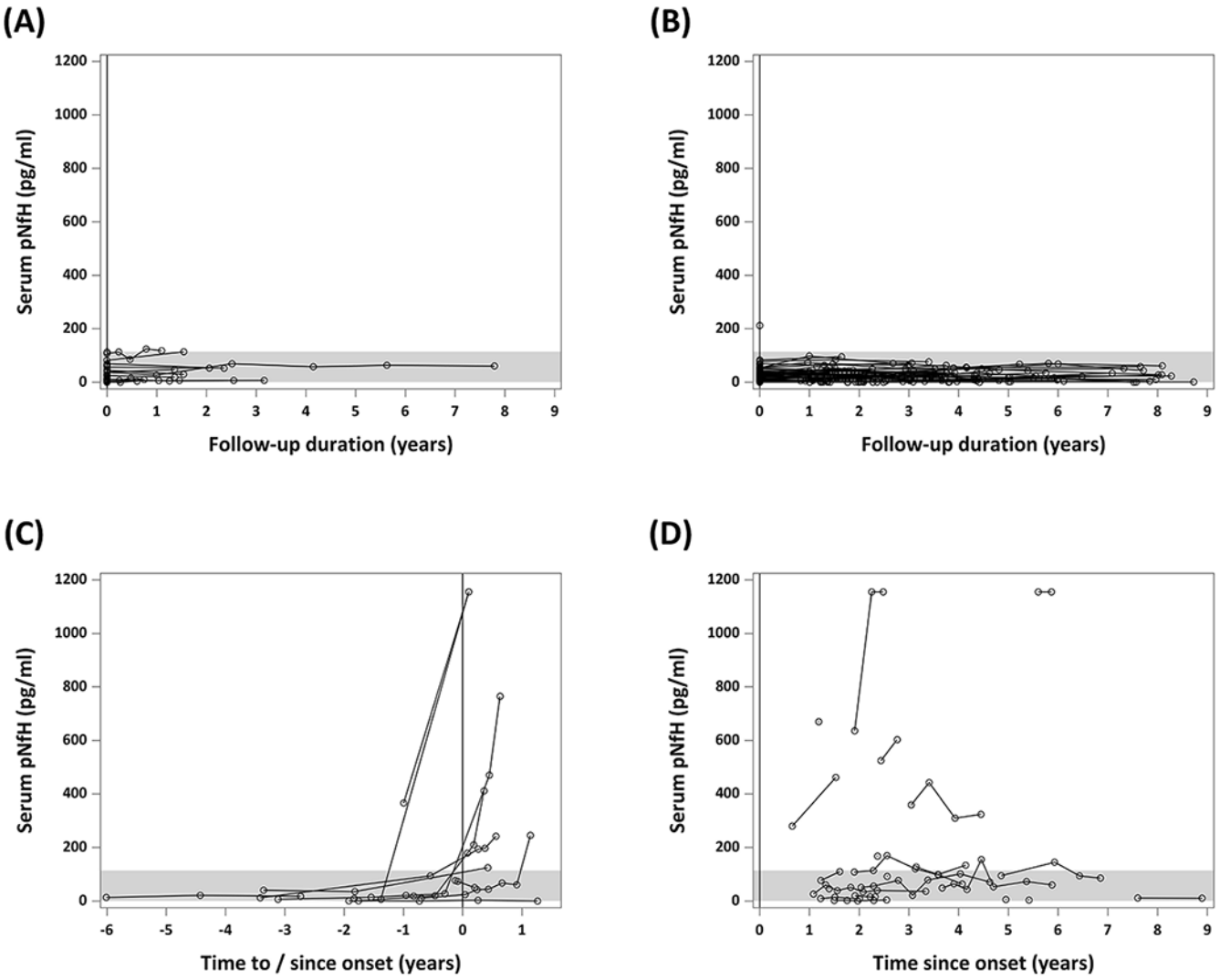

Figure 3. Longitudinal changes in serum pNfH concentrations (pg/ml)

(A) Controls; (B) at-risk individuals who remain pre-symptomatic throughout follow-up; (C) phenoconverters; and (D) ALS patients. The x-axis in (A) and (B) shows years since baseline. The x-axis in (C) and (D) shows years to or since the onset of symptoms/signs, which is marked by the vertical dashed line at year = 0. ALS = amyotrophic lateral sclerosis; pNfH = phosphorylated neurofilament heavy. Shading indicates the 95th percentile of all available data among controls.

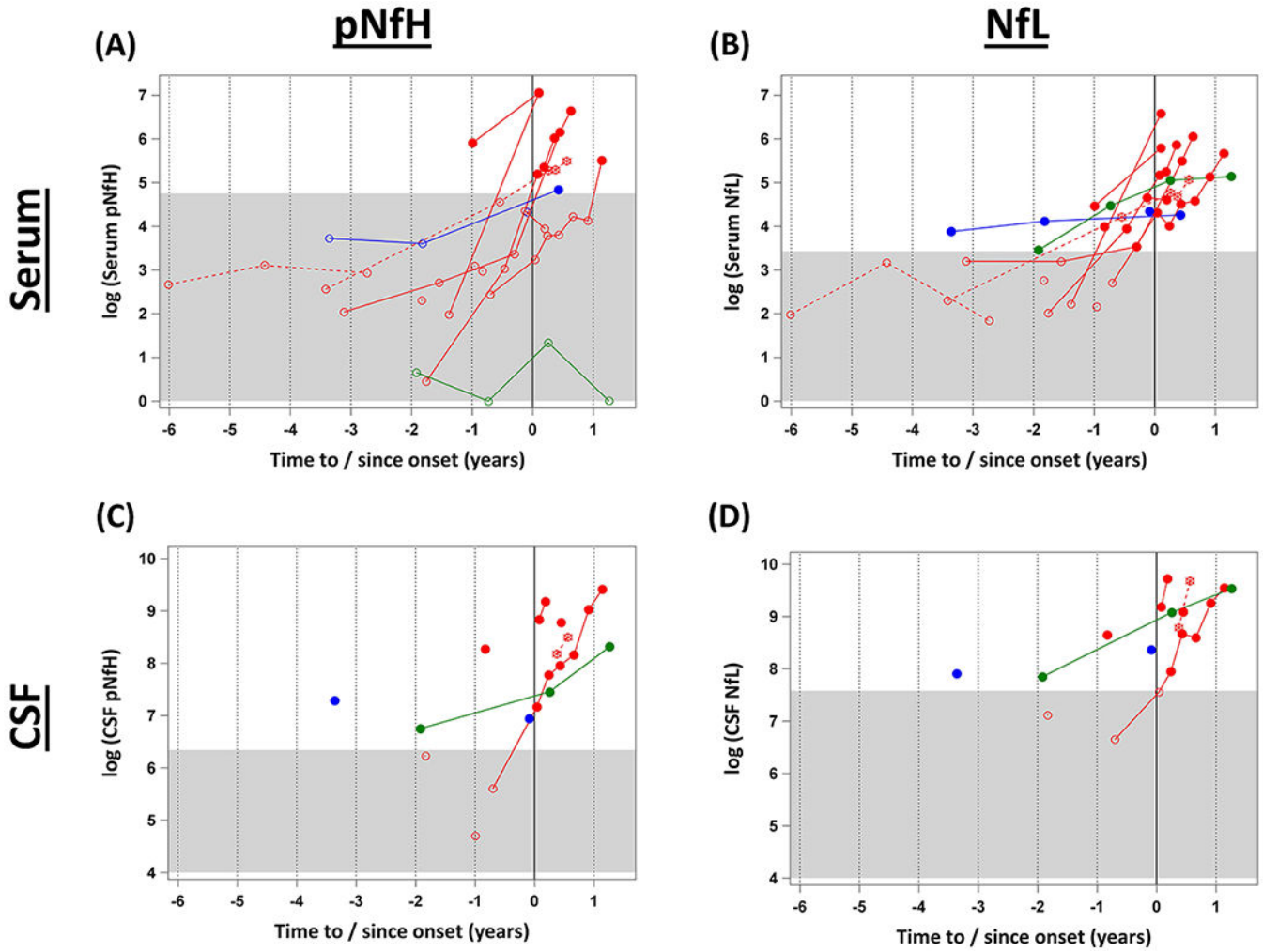

Figure 4. Longitudinal changes in serum NfL and pNfH among phenoconverters

(A) Serum pNfH; (B) serum NfL; (c) CSF pNfH; (d) CSF NfL each plotted on the natural logarithm scale. The x-axis shows years to or since the onset of symptoms/signs, which is marked by the vertical dashed line at year = 0. The gray area covers the range of values within the normative threshold, defined here as the 95th percentile of serum NfL or pNfH values observed in the control group. Colors indicate genotype, with *SOD1* A4V in solid red, *SOD1* non-A4V in dotted red, *FUS* in green, and *C9orf72* in blue. The closed circles mark the levels that are elevated above the normative threshold. ALS = amyotrophic lateral sclerosis; NfL = neurofilament light; pNfH = phosphorylated neurofilament heavy.

Table 1.

Study participant characteristics

		All Participants				Longitudinal Subset			
		Control (N=34)	**At-risk (N=79)**	**Converter (N=14)**	**Affected (N=22)**	**Control (N=13)**	**At-risk (N=55)**	**Converter (N=10)**	**Affected (N=16)**
# of collections	Median (Range)	(n/a)				2 (2-6)	3 (2-7)	3.5 (2-7)	3 (2-7)
Follow-up duration (years)	Median (Range)					1.5 (0.6-7.8)	3.7 (1.0-8.7)	2.7 (0.3-4.0)	1.0 (0.3-2.6)
Baseline age (years)	Mean ± SD (Range)	46.4 ± 11.4 (24.2-69.2)	44.8 ± 12.4 (18.9-77.0)	51.1 ± 12.2 (31.9-74.8)	59.2 ± 7.7 (45.6-75.8)	48.1 ± 11.9 (34.5-69.2)	47.0 ± 11.4 (18.9-67.6)	51.8 ± 11.7 (32.0-74.8)	59.8 ± 6.9 (45.6-67.7)
Male	N (%)	15 (44%)	28 (35%)	7 (50%)	12 (55%)	4 (31%)	19 (35%)	3 (30%)	9 (56%)
Genotype	*SOD1* A4V *SOD1* nonA4V *C9ORF72 HRE* Other Unknown	(n/a)	27 22 25 5 0	9 2 2 1 0	1 1 5 0 15	(n/a)	21 17 13 4 0	6 2 1 1 0	1 0 4 0 11
Site of onset	Bulbar Limbs Other Unknown	(n/a)		2 10 2 0	2 18 0 2	(n/a)		1 7 2 0	1 13 0 2
Baseline years since onset	Median (Range)			−1.6 (−6.0, −0.1)	2.2 (0.7, 7.6)			−1.8 (−6.0, −0.1)	2.0 (0.7, 7.6)
Baseline years since diagnosis	Median (Range)			−1.7 (−6.2, −0.3)	1.0 (0.1, 3.4)			−1.9 (−6.2, −0.3)	0.9 (0.1, 3.4)
Baseline ALSFRS-R	Mean ± SD (Range)			(n/a)	34.2 ± 7.8 (9-44) [a,b]			(n/a)	35.4 ± 8.5 (9-44) [a,c]
Baseline ΔFRS	Mean ± SD (Range)			(n/a)	0.53 ± 0.38 (0.14-1.45) [a]			(n/a)	0.49 ± 0.34 (0.14-1.31) [a]

Baseline = first visit at which serum sample was available (with or without contemporaneous CSF collection)

Follow-up duration = time between the participant's first and last serum sample included in this study

(n/a) = not applicable.

[a] Baseline ALSFRS-R not available for N=2

[b] Excluding N=1 with baseline ALSFRS-R=9, 35.5 ± 5.2 (27-44).

[c] Excluding N=1 with baseline ALSFRS-R=9, 37.4 ± 4.0 (31-44).

Table 2.

Serum and CSF pNfH concentration

		Baseline Visit Only				All Available Visits (Baseline & Follow Up)			
		Control	**At-risk**	**Converter [b]**	**Affected**	**Control**	**At-risk**	**Converter [c]**	**Affected**
Serum pNfH:	**Samples available:** **pNfH below LOD [a]:**	***N=34*** ***N=2***	***N=79*** ***N=6***	***N=14*** ***N=0***	***N=22*** ***N=0***	***59 visits*** ***3 visits***	***224 visits*** ***17 visits***	***42 visits*** ***2 visits***	***68 visits*** ***0 visit***
Original scale (pg/ml)	Median (Range)	22.9 (0.03-113.2)	20.6 (1.2-212.7)	12.7 (0.6-366.6)	84.7 (2.0-1,156)	25.7 (0.03-124.9)	22.1 (0.03-212.7)	43.4 (0.6-1,156)	75.1 (1.1-1,156)
Log-transformed [d]	Mean ± SD (Range)	2.9 ± 1.3 (0-4.7)	3.0 ± 1.0 (0.8-5.4)	2.8 ± 1.5 (0.4-5.9)	4.2 ± 1.7 (1.1-7.1)	3.1 ± 1.2 (0-4.8)	2.9 ± 1.0 (0-5.4)	3.9 ± 1.6 (0.4-7.1)	4.2 ± 1.5 (0.7-7.1)
CSF pNfH:	**Samples available [e]:**	***N=15***	***N=46***	***N=7***	***N=9***	***21 visits***	***116 visits***	***20 visits***	***23 visits***
Original scale (pg/ml)	Median (Range)	253 (24-1,321)	252 (109-612)	852 (109-3,900)	1,657 (639-11,418)	253 (24-1,321)	278 (73.7-1,137)	2,853 (109-12,244)	1,782 (639-11,418)
Log-transformed [d]	Mean ± SD (Range)	5.5 ± 0.9 (3.2-7.2)	5.5 ± 0.4 (4.7-6.4)	6.5 ± 1.2 (4.7-8.3)	7.6 ± 0.9 (6.5-9.3)	5.5 ± 0.8 (3.2-7.2)	5.6 ± 0.5 (4.3-7.0)	7.7 ± 1.3 (4.7-9.4)	7.6 ± 0.8 (6.5-9.3)

Baseline = first visit at which serum sample was available (with or without contemporaneous CSF collection)

N = number of participants. Visits = number of person-visits. LOD = limit of detection.

[a] These observations were assigned the lowest detectable value in analyses and figures, but excluded from the calculation of summary statistics presented in this table.

[b] All 14 converters were pre-symptomatic at baseline.

[c] The 42 person-visits in the converter group included visits from when participants were pre-symptomatic and after phenoconversion.

[d] Natural algorithm

[e] None of the pNfH concentrations was below the limit of detection. N=11 participants (N=1 control, N=7 at-risk, N=3 converters) had CSF pNfH only at follow-up visits. Their data are included in the "All Available Visits" columns but not the "Baseline Visit Only" columns.

Table 3.

Published data on neurofilaments in pre-symptomatic neurodegenerative diseases

Disease	Genotypes with Data Available	Biomarker	Matrix	Data Type	N	Compared to Controls	Pre-symptomatic Longitudinal Increase	Reference
HD	*HTT*	NfH	Plasma	Cross-sectional	29	No difference	--	Wild et al [24]
HD	*HTT*	NfL	Plasma	Cross-sectional	104	Higher	--	Byrne et al [8]
HD	*HTT*	NfL	Plasma	Longitudinal	97	--	Yes	Byrne et al [8]
HD	*HTT*	NfL	CSF	Cross-sectional	32	Higher	--	Vinther-Jensen et al [9]
AD	*PSEN1, APP*	NfL	Serum	Cross-sectional	19	Higher	--	Weston et al [4]
AD	*PESN1, APP*	NfL	Serum	Longitudinal	25	--	Yes	Weston et al [3]
AD	*PSEN1, PSEN2, APP*	NfL	Serum	Longitudinal	78	--	Yes	Preische et al [2]
FTD	*MAPT, GRN, C9orf72*	NfL	Serum	Cross-sectional	44	No difference	--	Meeter et al [7]
FTD	*CHMP2B*	NfL	CSF	Cross-sectional	6	No difference (after age adjustment)	--	Rostgaard et al [5]
FTD	*MAPT, GRN, C9orf72*	NfL	CSF	Cross-sectional	40	No difference	--	Meeter et al [7]
FTD	*MAPT, GRN, C9orf72*	NfL	CSF	Cross-sectional	8	No difference	--	Scherling et al [6]
FTD	*GRN*	NfL	CSF	Longitudinal	2	--	Unclear	Meeter et al [7]
FTD/ALS	*C9orf72*	NfL	CSF	Cross-sectional	25	No difference	--	Meeter et al [25]
FTD/ALS	*C9orf72*	NfL	CSF	Longitudinal	2	--	No	Meeter et al [25]
ALS	*C9orf72, SOD, TARDBP, FUS*	NfL	CSF	Cross-sectional	7	No difference	--	Weydt et al [26]
ALS	*C9orf72, SOD, TARDBP, FUS*	pNfH	CSF	Cross-sectional	9	No difference	--	Weydt et al [26]
ALS	*C9orf72, SOD, TARDBP, FUS*	NfL	Blood	Cross-sectional	11	No difference	--	Weydt et al [26]
ALS	*SOD1, FUS, C9orf72*	NfL	Serum	Cross-sectional	94	No difference	--	Benatar et al [11]
ALS	*SOD1, FUS*	NfL	Serum	Longitudinal	59	--	Yes	Benatar et al [11]
ALS	*SOD1, FUS, C9orf72*	NfL	CSF	Cross-sectional	63	No difference	--	Benatar et al [11]
ALS	*SOD1, FUS*	NfL	CSF	Longitudinal	32	--	Yes	Benatar et al [11]
ALS	*C9orf72*	pNfH	CSF	Cross-sectional	8	No difference	--	Poesen et al [27]

HD – Huntington's disease; AD – Alzheimer's disease; FTD – frontotemporal dementia; NfH – Neurofilament Heavy; pNfH – Phosphorylated neurofilament heavy; NfL – Neurofilament light; CSF – cerebrospinal fluid