Phosphorylated Neurofilament Heavy Chain: A Biomarker of Survival for *C9ORF72*-Associated Amyotrophic Lateral Sclerosis

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As potential treatments for *C9ORF72*-associated amyotrophic lateral sclerosis (c9ALS) approach clinical trials, the identification of prognostic biomarkers for c9ALS becomes a priority. We show that levels of phosphorylated neurofilament heavy chain (pNFH) in cerebrospinal fluid (CSF) predict disease status and survival in c9ALS patients, and are largely stable over time. Moreover, c9ALS patients exhibit higher pNFH levels, more rapid disease progression, and shorter survival after disease onset than ALS patients without *C9ORF72* expansions. These data support the use of CSF pNFH as a prognostic biomarker for clinical trials, which will increase the likelihood of successfully developing a treatment for c9ALS.

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Despite more than 50 large clinical trials in the past half-century, there is only 1 minimally effective treatment for amyotrophic lateral sclerosis (ALS), a devastating motor neuron disease. Nevertheless, since the discovery of *C9ORF72* G_4C_2 repeat expansions as the most common genetic cause of ALS,[1,2] significant advances have been made toward elucidating the mechanisms by which this mutation causes *C9ORF72*-associated ALS (c9ALS), and devising therapeutic strategies to combat them. Multiple lines of evidence place G_4C_2 repeat RNA and dipeptide repeat (DPR) proteins synthesized from these transcripts at the crux of c9ALS. Therapeutic strategies that target G_4C_2 RNA, such as antisense oligonucleotides (ASOs) and small molecules, reduce DPR protein levels, and mitigate other abnormalities caused by G_4C_2 transcripts in c9ALS models.[3–6]

As therapeutics for c9ALS are sought, we must address barriers in moving a treatment from bench to bedside, such as the lack of biomarkers to forecast disease progression and confirm target engagement in clinical trials. We recently established poly(GP) DPR proteins as a promising pharmacodynamic biomarker for G_4C_2 RNA-

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targeting ASOs (c9ASOs),[6] but forthcoming clinical trials for c9ASOs will also benefit from biomarkers that predict disease course. Phosphorylated neurofilament heavy chain (pNFH) and neurofilament light chain, which are released into the interstitial fluid during axonal injury and neurodegeneration, have emerged as putative prognostic biomarkers.[7] Levels of pNFH are elevated in cerebrospinal fluid (CSF) and blood from patients with ALS,[8–14] and some studies show that pNFH levels are associated with survival and/or indicators of disease progression.[8,9,11–13,15] The prognostic potential of pNFH, however, has yet to be specifically evaluated in c9ALS patients, a population that differs clinically and pathophysiologically from ALS patients without a *C9ORF72* repeat expansion.[16–19] We thus investigated pNFH as an urgently needed prognostic biomarker for c9ALS.

Subjects and Methods

Participants

An international sampling of CSF from *C9ORF72* expansion carriers (n = 135) and noncarriers with no known ALS- or frontotemporal dementia (FTD)-linked mutation (n = 107) was used. Samples were obtained from asymptomatic *C9ORF72* expansion carriers, healthy individuals, and clinically symptomatic patients diagnosed with ALS, ALS with comorbid FTD (ALS-FTD), or FTD (Table). ALS patients met El Escorial criteria for this diagnosis.[20] Diagnosis of FTD was obtained through established guidelines[21–24] and supported by neuropsychological testing and, in autopsied patients, by pathologically verified frontotemporal lobar degeneration. Our cohort comprised a collection of existing samples from multiple biobanks and included samples collected specifically for studies on neurofilaments. Longitudinally collected CSF was available from 37 *C9ORF72* expansion carriers and 17 noncarriers. Written informed consent was obtained from all participants or their legal next of kin if they were unable to give written consent, and biological samples were obtained with ethics committee approval.

Sample Collection

The standard operating procedures for the collection, processing, and storage of CSF were generally consistent among sites. In brief, CSF was collected in polypropylene tubes by lumbar puncture (LP) and immediately placed on ice. With the exception of samples from 3 groups, the CSF was spun at low speed at 4°C within 30 minutes of collection to pellet any cellular debris. Samples were aliquoted before storing at −80°C.

pNFH Analysis

The previously described Meso Scale Discovery immunoassay used for this study employs a mouse antihuman pNFH antibody and a sulfo-tagged polyclonal anti-pNFH antibody as the capture and detection antibodies, respectively, and a purified bovine pNFH calibrator.[25] The assay has been analytically validated as a laboratory-developed test in the Iron Horse Clinical Laboratory Improvement Amendments–certified laboratory. Samples tested in duplicate have a coefficient of variation < 10%. The intra- and interday precision of the assay is also <10%. Reagents and quality control samples were transferred to Mayo Clinic Jacksonville, where all CSF samples were tested at an 8-fold dilution, to establish commutability between the two sites.

Statistical Analysis

ALS patient functional status was determined using the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R). For cross-sectional studies, the disease progression score was calculated using the equation: (48 − ALSFRS-R score at baseline)/disease duration in months from disease onset to baseline LP.[8] For longitudinal studies, we used: (48 − ALSFRS-R score at last LP)/disease duration in months from disease onset to last LP.

Comparisons of pNFH levels across disease groups, and associations of pNFH with disease progression score or survival since disease onset, were conducted separately for *C9ORF72* expansion carriers and noncarriers, as described below. Our primary analyses also included comparing pNFH levels, disease progression scores, and survival after disease onset between *C9ORF72* expansion carriers and noncarriers. Given our 9 primary analyses, a Bonferroni adjustment was made and $p \leq 0.0056$ was considered statistically significant.

For regression analyses, pNFH values were log-scaled, and a square root transformation was applied to the disease progression scores due to their skewed distributions.

pNFH levels were compared among disease groups (asymptomatic/healthy, ALS/ALS-FTD, FTD) using multivariate linear regression (MLR) models adjusted for age at LP and gender. Given a statistically significant ($p \leq 0.0056$) difference among groups, post hoc pairwise comparisons were made, with $p \leq 0.0167$ considered significant after Bonferroni correction. The ability of pNFH to discriminate between disease groups was examined by estimating the area under the receiver operating characteristic (ROC) curve.

Associations of pNFH levels in ALS/ALS-FTD patients with disease progression scores were evaluated using MLR models adjusted for age at disease onset, gender, and onset site. Additional adjustment for disease group (ALS or ALS-FTD) was made only in *C9ORF72* mutation carriers, as only 2 noncarriers had ALS-FTD.

Associations of pNFH levels in ALS/ALS-FTD patients with survival after disease onset were examined using multivariate Cox proportional hazards regression models. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated, and censoring occurred at the date of last follow-up. The multivariate model for *C9ORF72* mutation carriers was adjusted for age at disease onset, gender, disease group, and onset site. For non–mutation carriers, we adjusted only for age at disease onset and onset site due to the smaller number of deaths in this subgroup.[26] We additionally estimated the concordance index (c-index) with and without pNFH in a given Cox model to provide an alternative measure of the predictive ability of pNFH; a c-index of 0.5 indicates

predictive ability equal to that obtained by chance, and a c-index of 1.0 indicates perfect predictive ability.

Comparisons of pNFH between ALS patients with a *C9ORF72* mutation and those without were made using MLR models adjusted for age at LP, gender, onset to LP, and onset site. Disease progression scores were compared using MLR models adjusted for age at disease onset, gender, and onset site. Comparisons of survival after disease onset were made using multivariate Cox regression models adjusted for age at disease onset, gender, and onset site.

For our secondary analyses, $p \leq 0.05$ was considered statistically significant. The association of pNFH with poly(GP) levels in *C9ORF72* repeat expansion carriers, the latter measured as part of a recently published study,[6] was examined using a linear regression model. We also evaluated whether pNFH levels change over time in subjects with longitudinally collected CSF. The slope from a linear regression model (where pNFH was the response and time after the baseline pNFH measure was the predictor variable) was calculated separately for each patient, and these slopes were tested for difference from a value of zero (indicating no change in pNFH levels over time) using a 1-sample *t* test.

All statistical tests were 2-sided, and analyses were performed using SAS (v9.2; SAS Institute, Cary, NC) and R Statistical Software (v2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

Results

Evaluating CSF pNFH in C9ORF72 Mutation Carriers

To investigate the prognostic utility of pNFH in c9ALS patients with or without comorbid FTD, the prevalence of which is increased in *C9ORF72* expansion carriers,[16,17] we evaluated associations between pNFH levels and disease status, progression, and survival after disease onset using CSF from 86 c9ALS patients (14 with comorbid FTD [c9ALS-FTD]) and 28 asymptomatic individuals. Included for comparison was CSF from 21 c9FTD patients (see Table).

Compared to asymptomatic *C9ORF72* expansion carriers, CSF pNFH levels were higher in patients with c9ALS/c9ALS-FTD ($p < 0.0001$) or c9FTD ($p = 0.0004$; see Fig 1A). pNFH levels discriminated between c9ALS/c9ALS-FTD patients and asymptomatic individuals with an almost perfect area under the ROC curve of 0.996 (95% CI = 0.989–1.000). A cutoff value of 176pg/ml produced a sensitivity of 98.8% and a specificity of 96.4% (see Fig 1B). In addition, pNFH levels were higher in c9ALS/c9ALS-FTD compared to c9FTD ($p < 0.0001$), with pNFH levels discriminating between c9ALS/c9ALS-FTD patients and c9FTD patients with an area under the ROC curve of 0.899 (95% CI = 0.808–0.990).

In c9ALS/c9ALS-FTD patients, there was a positive correlation between pNFH levels and disease progression score calculated using the ALSFRS-R ($p = 0.012$, n = 63; see Fig 1C), but this did not reach statistical significance (set at $p \leq 0.0056$) after correction for multiple testing.

Survival data were available for 81 of the 86 c9ALS/c9ALS-FTD patients; the median length of follow-up after disease onset was 3.3 years (range = 1.0–11.7 years), and 59 patients (72.8%) died. A strong association was observed between higher pNFH levels and shorter survival after disease onset (HR [per each doubling of pNFH] = 2.16, 95% CI = 1.47–3.16, $p < 0.0001$; see Fig 1D). Further illustrating the ability of pNFH levels to predict survival after disease onset, the model c-index was 0.66 when adjusting for age at disease onset, gender, disease group, and onset site; it improved to 0.72 when pNFH was additionally included in the model.

Evaluating CSF pNFH in Non–C9ORF72 Mutation Carriers

We examined the same associations as above in individuals without a *C9ORF72* mutation: 37 healthy subjects, 45 ALS patients (2 with comorbid FTD), and 25 FTD patients (see Table).

CSF pNFH levels were significantly higher in ALS/ALS-FTD patients compared to healthy individuals or FTD patients ($p < 0.0001$; see Fig 1E), but not significantly different between FTD patients and controls ($p = 0.054$). pNFH levels discriminated between ALS/ALS-FTD patients and healthy individuals with an area under the ROC curve of 0.926 (95% CI = 0.861–0.991); a cutoff value of 245pg/ml produced a sensitivity of 89.0% and a specificity of 100.0% (see Fig 1F). pNFH levels discriminated between ALS/ALS-FTD patients and FTD patients with an area under the ROC curve of 0.867 (95% CI = 0.776–0.957).

pNFH levels in ALS/ALS-FTD patients did not associate with disease progression score ($p = 0.12$, n = 36; see Fig 1G).

Survival data were available for 40 of the 45 ALS/ALS-FTD patients; the median length of follow-up after disease onset was 5.0 years (range = 1.4–20.0 years), and 18 patients (45.0%) died. A strong association was observed between higher pNFH levels and shorter survival after disease onset (HR [per each doubling in pNFH] = 3.04, 95% CI = 1.58–6.59, $p = 0.002$; see Fig 1H). Again demonstrating the ability of pNFH to predict survival after disease onset, the model that was adjusted for only age at disease onset and onset site had a c-index of 0.61, which increased to 0.77 when pNFH was also included in the model.

c9ALS Patients Exhibit Higher pNFH Levels and Disease Progression Scores, and Shorter Survival, Compared to ALS Patients without a C9ORF72 Expansion

We next compared indicators of disease progression between ALS patients with and without *C9ORF72* expansions (see Fig 1I, J and K). Given the increased prevalence of FTD in ALS patients with a *C9ORF72* expansion (14 of 86 patients [16.3%]) versus those without (2 of 45 patients [4.4%]), ALS-FTD patients were omitted from these analyses.

CSF pNFH levels ($p < 0.0001$) and disease progression scores ($p = 0.003$) were significantly higher in

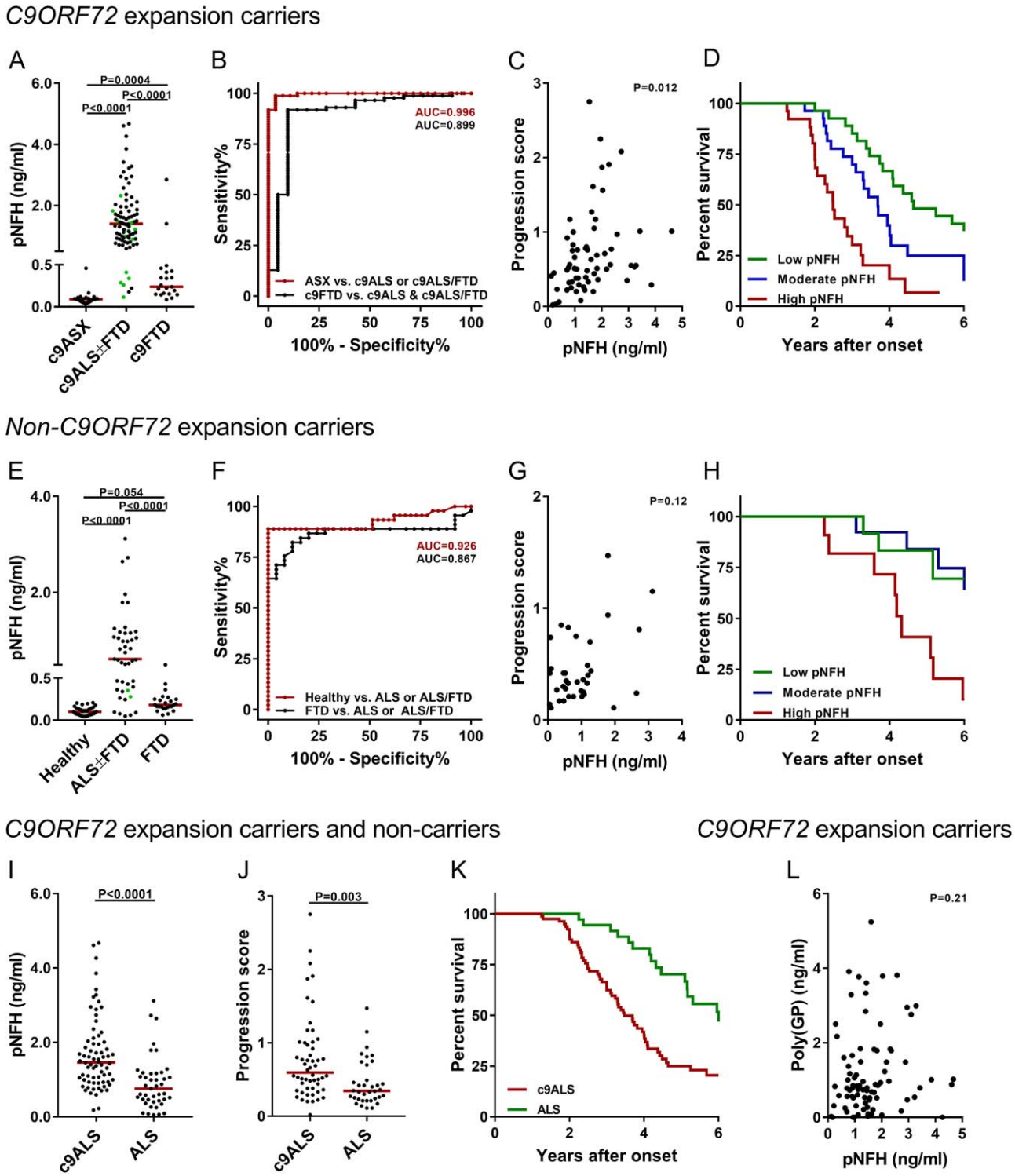

FIGURE 1

c9ALS patients compared to patients without the expansion. Similarly, there was strong evidence of shorter survival after disease onset for patients with c9ALS compared to those without the expansion (HR = 4.08, 95% CI = 2.24–7.43, $p < 0.0001$).

We recently reported that CSF poly(GP) levels, although a promising pharmacodynamic biomarker, do not associate with survival after disease onset in c9ALS/c9ALS-FTD.[6] Consistent with these findings, poly(GP) levels did not associate with pNFH levels ($p = 0.21$; see Fig 1L).

Longitudinal pNFH Measurements

Longitudinally collected CSF was available from 37 *C9ORF72* expansion carriers and 17 noncarriers. The median length of time between the first and last pNFH measurement was 12.9 months (range = 4.4–22.6 months) and 5.8 months (range = 3.0–6.7 months) for *C9ORF72* expansion carriers and noncarriers, respectively. There was no evidence of a change in CSF pNFH levels over time in *C9ORF72* expansion carriers ($p = 0.75$), and the rate of change in CSF pNFH did not differ between asymptomatic individuals and patients with c9ALS or c9ALS-FTD ($p = 0.83$; Fig 2A). A similar lack of change in CSF pNFH over time was observed in ALS patients without a *C9ORF72* expansion ($p = 0.80$; see Fig 2B). There was no significant difference in the rate of change in CSF pNFH between faster and slower progressors in patients with a *C9ORF72* expansion ($p = 0.33$), without an expansion ($p = 0.16$), or combined ($p = 0.094$).

Discussion

Clinical trials for ALS are hampered by the lack of biomarkers to monitor target engagement and predict disease course. The latter frequently results in clinical trial treatment groups with significantly different proportions of fast and slow progressors, thereby greatly increasing the number of patients needed to detect a treatment effect. With therapeutics for c9ALS being intensely investigated, and clinical trials for c9ASOs approaching, we evaluated pNFH as a prognostic biomarker for c9ALS to overcome this barrier.

Our study revealed a strong association between higher CSF pNFH levels and shorter survival in ALS patients with or without a *C9ORF72* expansion, which is consistent with previous reports.[9,13] Given that the clinical course of disease can vary substantially—one study reports survival ranges of 0 to 7 years for c9ALS, and 3 to 38 years for ALS[16]—the ability of pNFH levels to predict survival could facilitate the assessment of treatment on prolonging life, and be used to stratify patients into more homogenous groups to improve clinical trial efficiency and the estimation of treatment outcomes.

We also noted a positive correlation between pNFH levels and disease progression score in c9ALS and c9ALS-FTD patients, but this was not significant after adjustment for multiple testing, perhaps because of lower power due to missing data for some patients, or a lack of precision in the disease progression score, which depends on a functional rating that is subject to bias.

That ALS patients with *C9ORF72* expansions had significantly higher pNFH levels than patients without

FIGURE 1: Evaluation of cerebrospinal fluid (CSF) phosphorylated neurofilament heavy chain (pNFH) in *C9ORF72* repeat expansion carriers and noncarriers. (A–H) pNFH levels were measured in CSF from individuals with a *C9ORF72* repeat expansion (A–D) and those without an expansion (E–H). (A) Scatter plot showing that CSF pNFH levels were higher in patients with *C9ORF72*-associated amyotrophic lateral sclerosis with or without comorbid frontotemporal dementia (c9ALS ± FTD, n = 86), or patients with c9FTD (n = 21), compared to asymptomatic *C9ORF72* repeat expansion carriers (c9ASX, n = 28). Patients with c9ALS-FTD are represented by green circles. (B) Receiver operating characteristic (ROC) curves showing the ability of CSF pNFH levels to discriminate between patients with c9ALS or c9ALS-FTD (n = 86) and either asymptomatic *C9ORF72* repeat expansion carriers (n = 28) or patients with c9FTD (n = 21). AUC = area under the curve. (C) Association of CSF pNFH levels and disease progression scores in patients with c9ALS or c9ALS-FTD (n = 63). (D) Survival after disease onset according to CSF pNFH levels for c9ALS and c9ALS-FTD patients (n = 81). For ease of presentation, pNFH levels were divided into a 3-level categorical variable based on sample tertiles (Low ≤ 1.0980ng/ml, Moderate = 1.0981–1.6860ng/ml, High > 1.6860ng/ml). (E) Scatter plot showing that CSF pNFH levels among individuals without a *C9ORF72* repeat expansion were higher in ALS patients with or without comorbid FTD (n = 45) compared to healthy individuals (n = 37) or patients with FTD (n = 25). Patients with ALS-FTD are represented by green circles. (F) ROC curves showing the ability of CSF pNFH levels to discriminate between patients with ALS or ALS-FTD (n = 45) and either healthy individuals (n = 37) or patients with FTD (n = 25) without a *C9ORF72* expansion. (G) CSF pNFH levels did not associate with disease progression scores in patients with *C9ORF72*-negative ALS or ALS-FTD (n = 36). (H) Survival after disease onset according to CSF pNFH levels for ALS and ALS-FTD patients without a *C9ORF72* expansion (n = 40). For presentation purposes, pNFH levels were divided into a 3-level categorical variable based on sample tertiles (Low ≤ 0.4660ng/ml, Moderate = 0.4661–1.0540ng/ml, High > 1.0540ng/ml). (I) Comparison of CSF pNFH levels between patients with c9ALS (n = 72) and *C9ORF72*-negative ALS (n = 43). (J) Comparison of disease progression scores between patients with c9ALS (n = 54) and *C9ORF72*-negative ALS (n = 36). (K) Comparison of survival after disease onset between patients with c9ALS (n = 68) and *C9ORF72*-negative ALS (n = 38). (L) CSF pNFH levels did not associate with CSF poly(GP) levels in patients with c9ALS or c9ALS-FTD (n = 86). Straight lines in panels A, E, I, and J represent the median.

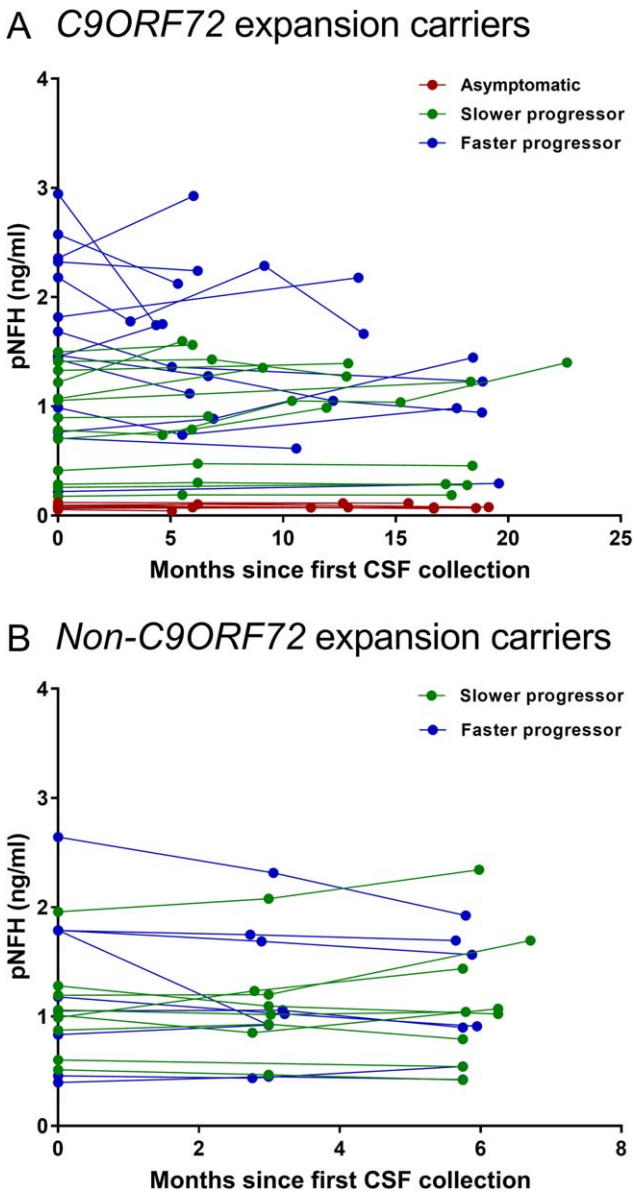

FIGURE 2: Longitudinal evaluation of cerebrospinal fluid (CSF) phosphorylated neurofilament heavy chain (pNFH) in *C9ORF72* repeat expansion carriers and noncarriers. pNFH levels were measured in CSF collected longitudinally from *C9ORF72* repeat expansion carriers and noncarriers. Subjects are color-coded based on their disease progression score, with patients having a score higher than the median (0.54 and 0.45 for *C9ORF72* expansion carriers and noncarriers, respectively) considered faster progressors, whereas those with a score equal to or lower than the median are considered slower progressors. (A) Among the expansion carriers, 10 were asymptomatic, 20 were patients with *C9ORF72*-associated amyotrophic lateral sclerosis (c9ALS), and 7 were patients with c9ALS and comorbid frontotemporal dementia. For these 37 subjects, the median length of time between the first and last pNFH measurement was 12.9 months (range = 4.4–22.6 months); 25 subjects had 2 measurements, 9 subjects had 3 measurements, 2 subjects had 4 measurements, and 1 subject had 5 measurements. (B) For the 17 ALS patients without a *C9ORF72* expansion, the median length of time between the first and last pNFH measurement was 5.8 months (range = 3.0–6.7 months), and CSF pNFH was measured either 2 times (n = 2) or 3 times (n = 15).

this mutation presumably reflects increased neuronal injury. c9ALS patients develop greater brain atrophy, particularly in extramotor regions, compared to ALS patients without a *C9ORF72* expansion.[17,18] This diffuse spread of degeneration may account for the more rapid disease progression and shorter survival of c9ALS patients, as observed herein and by others.[16–19]

We show that CSF pNFH levels are largely stable over time. These findings from our relatively large cohort of ALS patients are consistent with those from a study examining 11 ALS patients longitudinally.[9] This stability of pNFH could facilitate its use as a pharmacodynamic biomarker for drugs that mitigate axonal injury and neurodegeneration. Additional longitudinal studies spanning

TABLE Patient Characteristics according to *C9ORF72* Repeat Expansion Status and Disease Group

Characteristic	*C9ORF72* Repeat Expansion Carriers			Non-*C9ORF72* Repeat Expansion Carriers		
	Asymptomatic, n = 28	c9ALS or c9ALS-FTD, n = 86	c9FTD, n = 21	Healthy, n = 37	ALS or ALS-FTD, n = 45	FTD, n = 25
Age at LP, yr	43 (28, 63)	59 (36, 76)	63 (45, 77)	60 (23, 85)	60 (26, 90)	67 (59, 84)
Age at disease onset, yr	N/A	57 (34, 74)	61 (44, 76)	N/A	57 (25, 87)	65 (39, 80)
Gender, male	8 [28.6%]	53 [61.6%]	11 [52.4%]	13 [35.1%]	30 [66.7%]	17 [68.0%]
Disease onset to LP, mo	N/A	23 (0, 132)	47 (0, 103)	N/A	27 (6, 204)	51 (6, 243)
Onset site						
Bulbar	N/A	28 [33.7%]	N/A	N/A	10 [23.3%]	N/A
Limb	N/A	51 [61.4%]	N/A	N/A	31 [72.1%]	N/A
Other	N/A	4 [4.8%]	N/A	N/A	2 [4.7%]	N/A
ALSFRS-R score	N/A	36 (7, 46)	N/A	N/A	37 (19, 45)	N/A
Disease progression score	N/A	0.5 (0.0, 2.8)	N/A	N/A	0.3 (0.1, 1.5)	N/A
pNFH level, pg/ml	89 (35, 459)	1,406 (117, 4,671)	238 (86, 2,848)	100 (45, 209)	621 (50, 3,119)	181 (61, 502)

The sample median (minimum, maximum) is given for continuous variables. Information was unavailable for some subjects regarding age at LP (n = 1 c9ALS, n = 1 ALS, n = 1 FTD), age at disease onset (n = 1 c9FTD), disease onset to LP (n = 1 c9FTD, n = 1 FTD), onset site (n = 1 c9ALS, n = 2 c9ALS-FTD, n = 2 ALS-FTD), ALSFRS-R score (n = 17 c9ALS, n = 5 c9ALS-FTD, n = 7 ALS, n = 2 ALS-FTD), and disease progression score (n = 18 c9ALS, n = 5 c9ALS-FTD, n = 7 ALS, n = 2 ALS-FTD). CSF samples were collected at the following institutions: Emory University School of Medicine, National Institutes of Health, Mayo Clinic Jacksonville, University of Miami, IRCCS Istituto Auxologico Italiano, University of Milan, Maggiore Hospital (Crema), Massachusetts General Hospital, University of Massachusetts Medical School, Barrow Neurological Institute, University of Pittsburgh Medical Center, University of Pennsylvania, Washington University School of Medicine, and University Hospital Mútua de Terrassa.

ALS = amyotrophic lateral sclerosis; ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; c9 = *C9ORF72*-associated; FTD = frontotemporal dementia; LP = lumbar puncture; N/A = not applicable; pNFH = phosphorylated neurofilament heavy chain.

asymptomatic and symptomatic phases of disease would be useful in determining at what point pNFH levels begin to rise—information that could inform when best to initiate treatment.

Overall, we established the prognostic potential of CSF pNFH for c9ALS. These findings, together with our discovery of a pharmacodynamic biomarker for G_4C_2 RNA-targeting therapies,[6] will increase the likelihood of successfully developing a treatment for c9ALS.

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Author Contributions

K.B.B., T.F.G., A.J., and L.P. contributed to the conception and design of the study. T.F.G., L.M.D., M.G.H., N.N.D., J.W., T.M.M., P.P., J.Q.T., M.G., J.D.B., W.T.H., A.R., M.B., V.S., J.D.G., M.K.F., and K.B.B. contributed to the acquisition and analysis of data. T.F.G. and M.G.H. drafted the text and prepared figures. Members of the *C9ORF72* Neurofilament Study Group (Mercedes Prudencio, Ana M. Caputo, Pamela Desaro, Carla Palmucci, Amelia Robertson, Yuping Song, Beth K. Rush, Otto Pedraza, David Lacomis, Shafeeq Ladha, Christina N. Fournier, Leo F. McCluskey, Lauren B. Elman, Jon B. Toledo, Jennifer D. McBride, Cinzia Tiloca, Claudia Morelli, Barbara Poletti, Federico Verde, Alessandro Prelle, Jennifer Jockel-Balsarotti, Rosa Rademakers, Bruce L. Miller, Adam L. Boxer, Howard J. Rosen, Robert H. Brown, Bradley F. Boeve, Robert Bowser, Monica Diez, Miquel Aguilar, Jeffrey D. Rothstein, Bryan J. Traynor) participated in the collection of patient samples and data.

Potential Conflicts of Interest

A.J. receives a salary from, and holds stock options with, Iron Horse Diagnostics. T.F.G. and L.P. have a U.S. patent on methods and materials for detecting *C9ORF72*-associated ALS and FTD using poly(GP) proteins (European patent filed).

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