Author/s:
Parkin, GM;Thomas, EA;Corey-Bloom, J

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Mapping neurodegeneration across the Huntington's disease spectrum: a five-year longitudinal analysis of plasma neurofilament light

Georgia M. Parkin,[a,c,*] Elizabeth A. Thomas,[b,c] and Jody Corey-Bloom[a]

[a]Department of Neurosciences, University of California San Diego, San Diego, 92093, CA, USA
[b]Department of Neurobiology and Behavior, University of California Irvine, Irvine, 92697, CA, USA
[c]Institute for Interdisciplinary Salivary Bioscience Research, University of California Irvine, Irvine, 92697, CA, USA

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Summary

Background Neurofilament light (NfL) has previously been highlighted as a potential biomarker for Huntington's Disease (HD) using cross-sectional analyses. Our study aim was to investigate how longitudinal trajectories of plasma NfL relate to HD disease stage.

Methods 108 participants [78 individuals with the HD mutation, and 30 healthy controls (HC)] were included in this study. Individuals with the HD mutation were categorised separately by both HD-Integrated Staging System (HD-ISS) (Study 1) and PIN score-Approximated Staging System (PASS) (Study 2) criteria. Plasma NfL trajectories were examined using Mixed Linear Modeling (MLM); associations with symptom presentation were assessed using Spearman's rho correlations.

Findings The MLM coefficients for disease stage (HD-ISS $\beta = 32.73$, $p < 0.0001$; PASS $\beta = 33.00$, $p < 0.0001$) and disease stage*time (HD-ISS $\beta = 7.85$, $p = 0.004$; PASS $\beta = 6.58$, $p = 0.0047$) suggest these are significant contributors to plasma NfL levels. In addition, the plasma NfL rate of change varied significantly across time (HD-ISS $\beta = 3.14$, $p = 0.04$; PASS $\beta = 2.94$, $p = 0.050$). The annualised rate of change was 8.32% for HC; 10.55%, 12.75% and 15.62% for HD-ISS Stage ≤1, Stage 2, and Stage 3, respectively; and 12.13%, 10.46%, 10.33%, 17.52%, for PASS Stage 0, Stage 1, Stage 2, and Stage 3, respectively. Plasma NfL levels correlated with the Symbol Digit Modalities Test (SDMT) in HD-ISS Stage ≤1, and both SDMT and Total Motor Score in Stage 3 (ps < 0.01).

Interpretation Our findings suggest that plasma NfL levels increase linearly across earlier disease stages, correlating with the cognitive SDMT measure. Thereafter, an increase or surge in plasma NfL levels, paired with correlations with both cognitive and motor measures, suggest a late acceleration in clinical and pathological progression.

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Keywords: Huntington's disease; Biomarkers; Neurofilament light; Plasma

Introduction

Huntington's Disease (HD) is a genetic, neurodegenerative disorder caused by abnormal (>35) trinucleotide (cytosine-adenine-guanine; CAG) repeat expansions in exon 1 of the *Huntingtin* gene; a disease for which there is currently no cure. Symptom presentation begins mid-life in a prodromal phase, often with the onset of subclinical cognitive difficulties, and behavioural and psychiatric symptoms, followed by subtle motor symptoms. The presence of unequivocal motor dysfunction is a hallmark requirement for the clinical motor diagnosis of manifest HD.[1] Currently, this clinical motor diagnosis is provided by a neurologist, following completion of the Unified Huntington's Disease Rating Scale (UHDRS), and indicates that the rater has ≥99% confidence that observed motor abnormalities are unequivocal signs of disease.[2]

While the separation of participants into 'pre-manifest' and 'manifest' HD categories is useful for clinical purposes, utilisation of these groupings is too simplified for the purpose of effecting research into progression across the full HD disease spectrum.

*Corresponding author. Department of Neurosciences, University of California, San Diego, La Jolla, CA, USA.
E-mail address: gparkin@health.ucsd.edu (G.M. Parkin).

Research in context

Evidence before this study

Huntington's Disease (HD) is a genetic, neurodegenerative disorder caused by abnormal (>35) trinucleotide (CAG) repeat expansions in exon 1 of the *Huntingtin* gene; a disease for which there is currently no cure. Symptom progression often occurs mid-life, with subsequent deterioration and ultimately a premature death 20 years after motor symptom onset. Recent efforts to standardise the description of the course of Huntington's disease have resulted in the development of the HD Integrated Staging System (HD-ISS), which categorises individuals with the HD mutation into disease progression cohorts for research purposes on the basis of quantitative neuroimaging, cognitive, and functional markers, while predictive models like the Prognostic Index Normed (PIN) score have also been introduced and adopted. We have previously shown that cross-sectional levels of plasma of neurofilament light (NfL), a neuronal marker associated with neurodegeneration, is associated with both HD-ISS category and PIN scores.

Added value of this study

This is a timely study that assesses longitudinal plasma NfL levels over five years of HD progression. We show that plasma NfL levels increase linearly across the earlier disease stages, correlating with a cognitive measure that has been shown to be very sensitive to disease progression (the Symbol Digit Modalities Test). Thereafter, a quadratic-like increase in plasma NfL levels, paired with correlations with both cognitive and motor measures, suggest a late acceleration in progression.

Implications of all the available evidence

Available evidence suggests that plasma NfL levels may have use in enriching specific stages of HD progression, beginning in Stage 1 of the HD-ISS – a time when individuals exhibit underlying basal ganglia pathology. Future research is needed to understand the full extent to which plasma NfL may be used as an early-stage marker of HD.

Consequently, efforts have been made by the HD research community to categorise individuals with the HD mutation into more accurate disease progression cohorts. To date, the main predictive models for disease burden and progression in HD clinical research–notably the several variations of disease burden score and CAG-Age Product (CAP) score, as well as the development of the Prognostic Index Normed (PIN) Score,[3] are based on the relationship between age and CAG repeat length.[4–7] Of note, variations of the CAP score were also recently harmonised to produce the CAP_{100} score, where CAP = 100 at the estimated age of clinical motor diagnosis.[5] However, none of these models included a wet biomarker variable, partly because such data wasn't available to them and partly because these models were developed in a hypothesis-driven way, with pre-defined variables.

Most recently, Tabrizi and colleagues proposed the Huntington's Disease Integrated Staging System (HD-ISS), which categorises individuals with the HD mutation into one of four stages based on the presence of pathophysiological and clinical symptom changes.[8] Specifically, Stage 0 defines individuals carrying the HD mutation, but without symptom presentation or detectable pathological change; Stage 1 delineates individuals exhibiting underlying basal ganglia pathology as measured by quantitative magnetic resonance imaging; Stage 2 demarcates individuals displaying a clinical phenotype evidenced by changes on the Symbol Digit Modalities Test (SDMT) and/or Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score (TMS); and Stage 3 describes individuals demonstrating functional decline characterised by changes on the UHDRS Total Functional Capacity (TFC) and/or Independence measures.[8] Notably, the HD-ISS also did not include any fluid biomarkers of disease progression, as no fluid biomarker met criteria for inclusion.

We have previously reported cross-sectional associations between levels of plasma neurofilament light (NfL)–a neuron-specific marker of axonal injury – and predicted years to clinical motor diagnosis (pCMD),[9] as well as between plasma NfL and PIN scores.[10] Our cross-sectional findings suggest that plasma NfL may have value in enriching HD-ISS Stage 1 sub-groups—individuals with underlying quantitative neuroimaging change but no clinical phenotype.[11] Unfortunately, although NfL has been examined frequently within the field of neurodegenerative disorders,[12–17] including HD,[18–20] longitudinal data is lacking. Indeed, to our knowledge, there is only one study of longitudinal plasma NfL levels in HD, and it was restricted to two collection time-points.[21] Our objective in this study was thus to examine the longitudinal trajectory of NfL levels in individuals with the HD mutation over a 5-year period, including an examination of how these levels relate to disease stage and symptom presentation across the HD spectrum.

Methods

Ethics

This longitudinal, observational study was conducted at the University of California, San Diego (UCSD) Huntington's Disease Society of America Centre of Excellence (HDSA CoE), following study approval by the UCSD Institutional Review Board (IRB) Committee

(IRB Protocol #170038) and in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects. All participants gave written informed consent prior to sample collection.

Participants

Individuals with the HD mutation, and healthy controls (HC), were recruited consecutively from the UCSD HDSA CoE and followed up for annual study visits for up to 5 years. Individuals with the HD mutation were considered eligible for inclusion in this study if they had a confirmed family history of HD, were not participating in a clinical trial, and had attended at least two annual study visits at the UCSD HDSA CoE. HC were considered eligible for inclusion in this study if they had a Montreal Cognitive Assessment (MoCA)[22] score of 26 or higher and had attended at least two annual study visits at the UCSD HDSA CoE. No other inclusion or exclusion criteria were applied. Each study visit included a blood draw, and completion of select cognitive (SDMT; score range 0–110,[23] MoCA,[22] Stroop test[24]), motor (UHDRS TMS; score range 1–124),[25] and functional (UHDRS TFC; score range 0–13)[12] measures. Scores from the Stroop wording reading test (SWR), SDMT, TFC and TMS were also incorporated into the composite UHDRS score (cUHDRS) as an additional measure of disease progression.[26] Gender was self-reported by the participant. Participants were recruited consecutively upon presentation to the UCSD HDSA CoE, irrespective of gender. Participants were included in this study based on the availability of longitudinal clinical data and matched blood samples, prior to the analysis of the blood samples for NfL or categorisation into HD-ISS stages, thereby limiting selection bias.

Individuals with the HD mutation were categorised into HD-ISS stages using published criteria,[8] and separately by PIN score as previously presented and outlined below.[11,27] The separation of participants into PIN score-determined disease stages, in addition to HD-ISS categories, allowed for the examination of Stage 0 and Stage 1 separately; these stages were combined for the analysis by HD-ISS to account for an absence of quantitative neuroimaging data. In addition, the PIN score itself serves as a predictive model for disease progression, applicable in various contexts, including the enrichment of populations for clinical trials and other clinical studies. Therefore, the use and presentation of PIN score thresholds for disease stage membership serves as an alternative staging system that accounts for age, CAG repeat length, SDMT and TMS score.

PIN score-approximated categorisation

We have previously shown that plasma NfL may have more use as a prognostic, early disease stage marker, rather than a marker of disease progression after clinical motor onset (manifest HD).[9,11] Therefore, we also wished to investigate plasma NfL level trajectories across early disease stages. Unfortunately, due to an absence of quantitative neuroimaging data, this was not possible using the HD-ISS. Therefore, we also categorised participants according to their PIN score as determined at their first study visit (herein referred to as PIN Score-Approximated Staging or PASS), as previously described.[11,27] A participant's PIN score was calculated using the previously published and validated formula,[3] where PIN = ((51 x TMS + (–34) x SDMT +7 x Age at visit x (CAG—34))—883)/1044. Briefly, participants with a PIN score ≤ –0.34 were categorised as Stage 0, those with a PIN score of > –0.34–0.60 as Stage 1, those with a PIN score of >0.60–2.31 as Stage 2 and those with a PIN score greater than 2.31 as Stage 3. These PIN score thresholds were previously published as those that provided the greatest (of all PIN score options) separation between participants of different HD-ISS categories.[27] We acknowledge that these PIN score thresholds have not otherwise been validated, and thus present this analysis as a second study, subsequent to that utilising the HD-ISS.

Plasma collection and NfL analysis

Blood was drawn by venipuncture into 2 ml lavender/EDTA tubes. EDTA/whole blood was mixed by inversion and centrifuged at 900*g* for 15 min. The supernatant was isolated, aliquoted into 1 ml aliquots, snap frozen and stored at –80 °C. Plasma levels of NfL were measured in duplicate using a Meso Scale Discovery (MSD; Rockville, MD) R-Plex Assay (Cat# F217X; researcher determined lower limit of detection: 2.3 pg/ml) as previously described.[9] Internal control inter-assay variation, prior to plate-to-plate normalisation, was 18%. After plate-to-plate normalisation, internal control inter-assay variation was <2%. Mean (SD) intra-participant, inter-assay (test/retest) variation, determined through the re-assay of a subset of 15 samples, was 24% (16%).

Statistics

Cohort characteristics

Analyses were conducted with GraphPad Prism version 8.4.2 (GraphPad Software, CA, USA) and SPSS Statistics (IBM, NY, USA). Comparisons of demographic data were conducted using participant baseline visit information only. Data distribution was assessed by Shapiro–Wilks test, and homogeneity of variance was assessed by Bartlett's test; correspondingly, participant age and CAP_{100} score were analysed by One-Way ANOVA, and all other variables (CAG repeat length, years of education, TMS, TFC, SDMT, MoCA, composite Unified Huntington's Disease Rating Scale (cUHDRS)) were assessed by Kruskal–Wallis test. Associations between plasma NfL and clinical measures were conducted using Spearman's rho and corresponding 95% confidence intervals (CI) were determined using the Bonnett and Wright test, due to the non-normal distribution of plasma NfL data. Bonferroni p-value correction of alpha was used to account for multiple correlation analyses. As

HD-ISS categorisation accounts for age, and plasma NfL levels did not correlate with CAG repeat length (as supported by data in this article and our previous publication[9]) additional adjustments for these covariates were not made.[3,8,27]

Plasma NfL trajectories

The analysis of plasma NfL trajectories was conducted using the Linear Mixed Modeling function on SPSS, which allows for timepoints missing at random. Briefly, participant follow-up visits were assigned a corresponding number of years (±6 months) since their first, or baseline, visit (time 0). For all steps of the analysis, plasma NfL levels were regressed against linear and quadratic terms for years since baseline visit (referred to as 'years since baseline', and 'quadtime', respectively) as covariates; the latter was included to accommodate for potential non-linear rates of change over time.[28] We first executed a Level 1 variance components model to determine whether between-participant clustering of NfL trajectories was evident, without factoring in participant grouping (such as HD-ISS category). For this model, 'participant ID' was keyed as a subject-grouping random effect.

Next, to verify the extent of the known relationship between plasma NfL levels and participant age within our sample, a Mixed Linear Model for Repeated Measures analysis with an autoregressive covariance structure at Level 1 (participant ID), and diagonal covariance structure for random main effects ('years since baseline', 'quadtime', under random effects subject grouping: 'participant ID'), was conducted, grouping participants by their decade of age at their first visit. Subsequently, the same model parameters were used, substituting participant gender, and then baseline HD-ISS or PASS category, for age bracket. Coefficient estimates were also investigated to compare the magnitude of association between each covariate and plasma NfL levels.

A complementary trajectory analysis using fractional polynomial regression was also conducted using NCSS software, in order to compare the Mixed Linear Modelling results to that determined using an 'untargeted' model-selection approach.[29]

Role of funders

The funding sources had no role in study design, conduct, analysis, interpretation or writing of the manuscript, or in the decision to submit the manuscript.

Results

Study 1

Mixed Linear Modeling of plasma NfL levels

An initial assessment of Level 1 covariance parameters (plasma NfL levels by participant ID and time-point) suggested a significant amount of between-participant variance in baseline plasma NfL levels and a need for Mixed Linear Modelling ($p < 0.0001$, intra-class coefficient (ICC) = 0.64).

Association between participant age bracket, and plasma NfL levels

Acknowledging the well-established association between plasma NfL levels and participant age,[30] we investigated the extent to which participant age bracket contributed to plasma NfL levels in our study. Participants were placed in age bracket bins based on their age at their first visit, with bins established in 10-year brackets starting at age 18 (18–27, 28–37, and so on). A Mixed Linear Model for Repeated Measures analysis, with a first-order autoregressive covariance structure at Level 1 (repeated measure: years since baseline), and diagonal covariance structure for random main effects (covariates: years since baseline, quadtime; under the random effects grouping: participant ID), considered the following fixed variables: years since baseline, quadtime, age bracket, and [age bracket]*[years since baseline]. In this model, neither years since baseline visit, nor the interaction between years since baseline and age decade, were significant contributors to plasma NfL levels. However, participant age decade itself was a significant contributor ($\beta = 21.96$, 95% CI = 13.68–30.25, $p < 0.0001$). When the same age decade analysis was conducted on the HC cohort, age decade similarly surfaced as a significant contributor to plasma NfL levels; however, with a smaller beta coefficient ($\beta = 12.80$, 95% CI = 6.96–18.63, $p < 0.0001$). When the same analysis was conducted, substituting participant gender for age decade, no significant contribution was detected (data not shown).

Association between years since baseline, HD-ISS, and plasma NfL

Cohort demographic and clinical assessment characteristics, with participants categorised by baseline HD-ISS grouping,[8] are summarised in Table 1. HD-ISS categories differed significantly with regard to age, CAG repeat, CAP_{100}, TMS, TFC, MoCA, SDMT and composite UHDRS (cUHDRS) scores. Cohort demographic and clinical assessment characteristics, by gender, are presented in Supplementary Table S1.

We hypothesised that HD-ISS categorisation, as a between-participant indicator of disease severity, may explain variance in participant baseline plasma NfL values, and contribute to plasma NfL trajectories. A Repeated Measures Mixed Linear Model analysis, with a first-order autoregressive covariance structure at Level 1 (repeated measure: years since baseline), and diagonal covariance structure for random main effects (covariates: years since baseline, quadtime, under the random effects subject grouping: participant ID),

n	Healthy Controls	HD-ISS			
		Stage ≤1	Stage 2	Stage 3	
	30	37	14	27	
					One-Way ANOVA p value
Age [years]	56.5, 46.0–61.3	47.0, 35.0–62.5	41.5, 36.0–62.3	56.0, 9.0–66.0	0.011
CAP_{100}	N/A	81.4, 55.5–98.3	98.1, 81.9–105.5	103.5, 90.6–120.2	<0.0001
					Kruskal–Wallis or Chi Square p value
Gender n[M/F], (%)	17/13, (57/43)	19/18, (51/49)	8/6, (57/43)	12/15, (44/56)	0.72
Education [years]	16.0, 12.0–18.0	16.0, 13.0–17.5	16.5, 14.5–18.0	15.0, 12.0–18.0	0.50
CAG Repeats	N/A	41.0, 40.0–42.0	43.5, 41.0–45.0	42.0, 40.0–42.0	0.0062
TMS	0.0, 0.0–0.0	1.0, 0.0–3.0	6.5, 2.8–13.8	33.5, 15.5–49.3	<0.0001
TFC	13.0, 13.0–13.0	13.0, 13.0–13.0	13.0, 13.0–13.0	9.0, 7.0–11.0	<0.0001
MoCA	28.0, 26.8–29.0	27.0, 26.0–29.0	26.5, 24.8–28.3	24.0, 20.0–26.0	<0.0001
SDMT	51.0, 45.0–56.3	52.0, 46.5–55.0	40.0, 31.3–52.5	25.0, 16.0–34.0	<0.0001
cUHDRS	17.5, 16.6–17.8	17.1, 16.3–18.5	15.6, 14.0–16.7	10.5, 7.8–12.6	<0.0001

CAP_{100}, standardised CAG-Age Product (CAP) score; cUHDRS, composite Unified Huntington's Disease Rating Scale; F, female; M, male; MoCA, Montreal Cognitive Assessment; TMS, Total Motor Score; TFC, Total Functional Capacity; SDMT, Symbol Digit Modalities Test. Healthy controls were not included in the analyses but are included in Table 1 as a qualitative indication of the general population range of values. P-values were determined using One-Way ANOVA, Kruskal–Wallis or Chi Square test as indicated, and relate to the comparison of HD-ISS Stage ≤1, Stage 2 and Stage 3.

Table 1: **Cohort characteristics (median, interquartile range) with HD-ISS categorisation.**

considered the following fixed variables: years since baseline, quadtime, HD-ISS, and [HD-ISS]*[years since baseline].

This model gave an intercept (time-point = 0, HD-ISS category = 0) of 18.64 pg/ml plasma NfL (95% CI = –9.043 to 46.32; p = 0.18). The coefficient for HD-ISS was β = 32.73 (95% CI = 19.57–45.88; p < 0.0001), suggesting that baseline plasma NfL values increased 32.73 pg/ml on average with each increase in HD-ISS category. While variation in plasma NfL across time for the overall population was not significant (p = 0.065), the [HD-ISS]*[years since baseline] coefficient was significant (β = 7.85; 95% CI: 2.53–13.17; p = 0.0043), suggesting that individuals in higher HD-ISS categories display greater increases in plasma NfL per time interval. The 'quadtime' coefficient was also significant (β = 3.15; 95% CI = 0.17–6.13; p = 0.039), indicating a variation in the plasma NfL rate of change across time. Correspondingly, the trajectory of plasma NfL with HD disease progression may be modelled by:

$$y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$

where $\beta_0 = 18.64$, $\beta 1 = 32.73$, $\beta 2 = 7.85$, $\beta 3 = 3.14$ and Xn is the corresponding value for participant i. In other words:

$$Estimates\ plasma\ NfL = 18.64 + 32.73\ [HD - ISS] + 7.85\ [HD - ISS * (Years\ since\ baseline)] + 3.14\ [(Years\ since\ baseline)^2]$$

A Mixed Linear Model analysis of the HC cohort, following the structure outlined above with the exclusion of HD-ISS and addition of age as a fixed effect covariate, determined that age (β = 1.19; 95% CI = 0.53–1.85; p = 0.00074), but not years since baseline, had a significant effect on HC plasma NfL levels (see Fig. 1c).

Plasma NfL trajectories within HD-ISS categories

Given the observed significant variation in plasma NfL rate of change over time, in addition to our previous cross-sectional findings suggesting that plasma NfL will likely be more useful in enriching sub-groups within a HD-ISS category,[11] we plotted longitudinal plasma NfL values by HD-ISS category (Fig. 1) and determined the corresponding linear equations, where y = plasma NfL value, and X = the number of years since baseline:

Stage ≤1 Y = 5.28X + 49.99

Stage 2 Y = 10.22X + 80.17

Stage 3 Y = 18.41X + 117.80

Healthy Controls Y = 3.52X + 42.24

Baseline and final assessed year plasma NfL values for each HD-ISS stage, as well as annual rate of change, were estimated using the above formulae and are presented in Table 2. Fig. 1 additionally shows the pCMD trajectories for the same participants, with the tail-end pCMD values for each stage also shown in Table 2.

Fractional polynomial regression analysis

Acknowledging that the use of a mixed linear model is limited by the preselection of only two possible polynomial models—linear and quadratic—and our

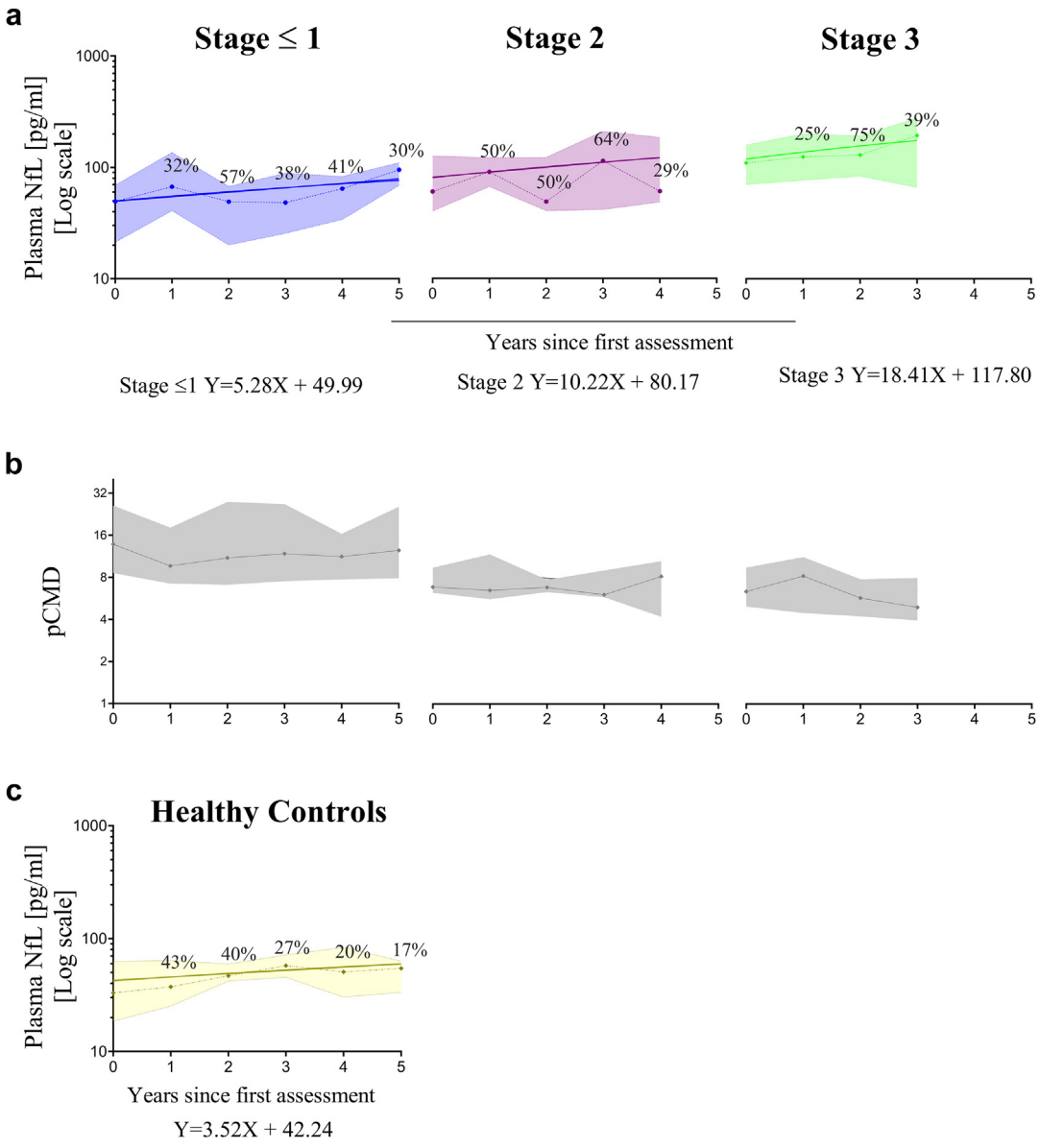

Fig. 1: A presentation of longitudinal NfL values by HD-ISS category (Stage ≤1 n = 37, Stage 2 n = 14, Stage 3 n = 27), determined at each participant's baseline visit (a), and the corresponding predicted years to clinical motor diagnosis (pCMD) values (b). Longitudinal plasma NfL values in healthy controls (n = 30) are presented in (Panel c). Symbols represent the median, and shaded areas represent the interquartile range. Percentage values provided represent the percentage of participants who contributed a value at that time-point (all time 0s = 100%). In (a) and (c), the coloured linear regression line corresponds to the linear regression equation below each graph. All biological measurements conducted with two technical replicates.

subsequent within-stage assessment assumes within-stage linearity, we also conducted a fractional polynomial analysis of plasma NfL levels across time, testing a total of 44 potential models. For the purpose of this analysis, to estimate the full trajectory of plasma NfL as shown in Fig. 1a, 'years since baseline' values were substituted in Stage 2 and 3 to follow sequentially from Stage ≤1, such that Stage 2 year 0 was considered six years since Stage ≤1 baseline, Stage 2 year 1 was seven years since first Stage ≤1 baseline and so on. A comparison of plasma NfL levels vs these adjusted years since first assessment, produced a highest ranked model of $Y = A0 + A1X + A2X^3$, with an R2 value of 0.30, where A0 = 50.21 (95% CI = 35.053–65.36, p < 0.0001), A1 = 4.045 (95% CI = –0.19 to 8.28, p = 0.061), and A2 = 0.022 (95% CI = –0.0011 to 0.045, p = 0.061) (Supplementary Figure S1). In other words:

	Category	Year	Estimated plasma NfL level (pg/ml)	PCMD	Annual ΔPlasma NfL (pg/ml)	Annual ΔPlasma NfL (%)
	Healthy Controls	Year 0	42.24	N/A	3.52	8.32
		Year 5	59.84	N/A		
HD-ISS	Stage ≤1	Year 0	49.99	16.33	5.28	10.55
		Year 5	76.37	13.95		
	Stage 2s	Year 0	80.17	9.04	10.22	12.75
		Year 4	121.05	6.86		
	Stage 3	Year 0	117.80	8.68	18.41	15.62
		Year 3	173.00	6.61		

Values were calculated based on the plasma NfL, and predicted years to clinical motor diagnosis (pCMD), trajectories presented in Fig. 1.

Table 2: **Tail-end median plasma NfL and predicted years to clinical motor diagnosis values, for HD-ISS categories.**

Estimated plasma NfL = 50.21 + 4.045 * (*Years since Stage ≤1 first baseline*)] + 0.022 [(*Years since Stage ≤ 1 baseline*)^3]

Estimated plasma NfL levels at the tail-end of each HD-ISS stage, as predicted using this equation, are presented in Supplementary Table S2. Conduct of the same analysis, with the addition of the HD-ISS variable, did not determine any models that could describe within- HD-ISS trajectories (Supplementary Figure S2, Supplementary Table S3). It is important to highlight, however, that these models do not account for a repeated measures design.

Plasma NfL correlations with clinical measures

We next examined whether variation in plasma NfL levels in each HD-ISS stage was associated with clinical presentation. Associations between plasma NfL levels and clinical variables were determined cross-sectionally, using data obtained at each participant's baseline visit (Table 3). In Stage ≤1, significant correlations that passed Bonferroni p-value correction were observed between plasma NfL and age, PIN score, CAP_{100}, pCMD, and SDMT. In Stage 2, no correlations were significant after Bonferroni p-value correction. In Stage 3, significant correlations that passed Bonferroni p-value correction were observed between plasma NfL, age, PIN score, CAP_{100}, pCMD, SDMT, TMS and cUHDRS. Correlations are presented graphically between plasma NfL and SDMT (Fig. 2a), TMS (Fig. 2b), and cUHDRS (Fig. 2c).

Study 2

Association between years since baseline, PASS, and plasma NfL

In the absence of quantitative neuroimaging data, use of HD-ISS categorisation is limited by the need to combine Stage 0 and Stage 1. We have previously shown that, when using PIN score approximation of HD-ISS categorisation (PASS), plasma NfL may be useful in

	Stage ≤1 (n = 37)	Stage 2 (n = 14)	Stage 3 (n = 27)
Demographic data			
Age [years]	**0.65 [0.38–0.81], <0.0001**	0.24 [−0.34 to 0.69], 0.40	**0.57 [0.22–0.79], 0.0018**
Education [years]	−0.10 [−0.41 to 0.23], 0.54	−0.01 [−0.54 to 0.52], 0.97	0.41 [0.02–0.69], 0.01
Disease data			
CAG	−0.08 [−0.39 to 0.25], 0.65	−0.35 [−0.75 to 0.24], 0.22	0.04 [−0.35 to 0.41], 0.85
PIN	**0.64 [0.37–0.81], <0.0001**	0.22 [−0.36 to 0.68], 0.45	**0.59 [0.23–0.80], 0.0014**
CAP_{100}	**0.67 [0.43–0.82], <0.0001**	0.26 [−0.33 to 0.71], 0.37	**0.66 [0.36–0.83], 0.00019**
pCMD	**−0.64 [−0.81 to −0.37], <0.0001**	−0.09 [−0.59 to 0.46], 0.76	**−0.44 [−0.71 to −0.05], 0.021**
Clinical data			
MoCA	−0.06 [−0.38 to 0.28], 0.75	−0.42 [−0.79 to 0.16], 0.13	−0.21 [−0.55 to 0.20], 0.31
SDMT	**−0.46 [−0.69 to −0.14], 0.0044**	−0.46 [−0.81 to 0.13], 0.10	**−0.57 [−0.79 to −0.21], 0.0020**
TMS	0.34 [0.01–0.60], 0.039	0.03 [−0.51 to 0.55], 0.92	**0.60 [0.26–0.81], 0.00088**
TFC	−0.35 [−0.61 to −0.02], 0.032	−0.24 [−0.69 to 0.34], 0.41	−0.24 [−0.57 to 0.16], 0.22
SWR	−0.13 [−0.44 to 0.20], 0.43	−0.40 [−0.78 to 0.19], 0.16	−0.39 [−0.68 to 0.001], 0.043
cUHDRS	−0.38 [−0.63 to −0.05], 0.021	−0.54 [−0.84 to 0.03], 0.047	**−0.60 [−0.81 to −0.26], 0.0085**

cUHDRS, composite Unified Huntington's Disease Rating Scale; MoCA, Montreal Cognitive Assessment; SDMT, Symbol Digit Modalities Test; SWR, Stroop Wording Reading; TFC, Total Functional Capacity; TMS, Total Motor Score. Bold values passed Bonferroni correction for multiple comparisons.

Table 3: **Association of plasma NfL levels, demographic and clinical measures byHD-ISS category (Spearman rho [95% CI], p value).**

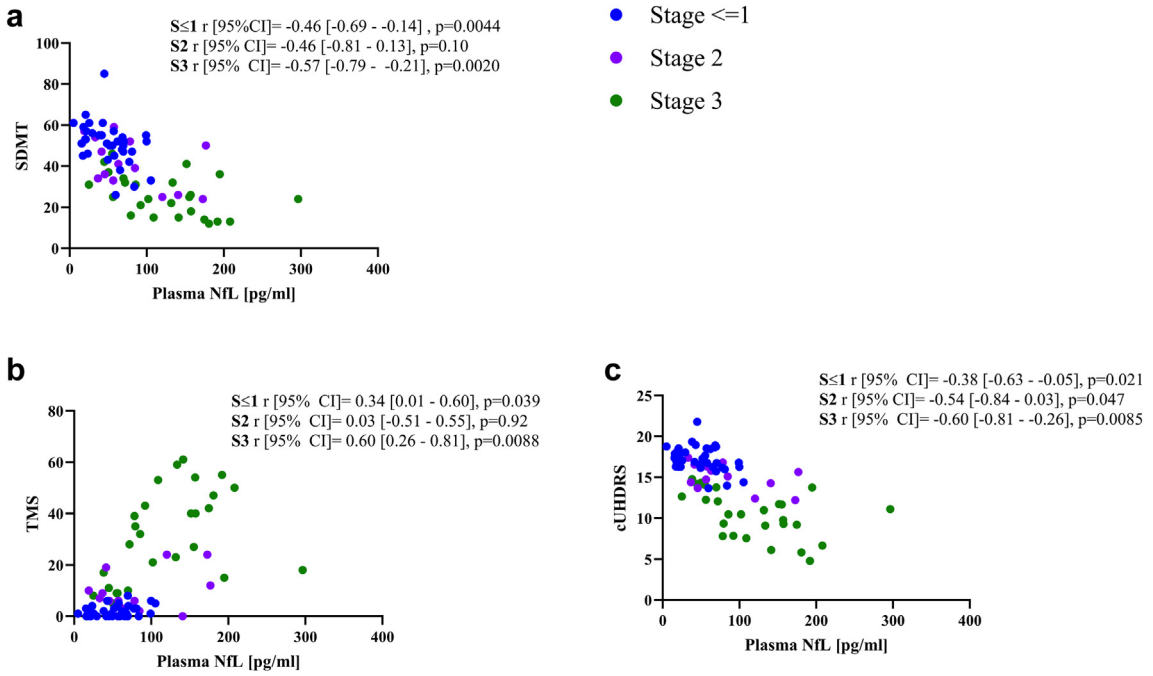

Fig. 2: Graphical presentation of associations between plasma NfL and clinical scores—Symbol Digit Modalities Test (SDMT) (a), Total Motor Score (TMS) (b), and composite Unified Huntington's Disease Rating Scale (cUHDRS) (c)–that passed Bonferroni p value correction. Data is grouped into HD-ISS Stage ≤1 (blue; n = 37), Stage 2 (purple; n = 14) and Stage 3 (green; n = 27). Values presented are Spearman's rho correlations and associated p values. All biological measurements conducted with two technical replicates.

enriching a Stage 1 subgroup.[11] We therefore repeated the MLM analysis, with participants categorised by PASS. Cohort characteristics for these alternately staged groups are presented in Table 4. Cohort characteristics, by gender, are presented in Supplementary Table S4.

A Repeated Measures Mixed Linear Model analysis, with a first-order autoregressive covariance structure at Level 1 (repeated measure: years since baseline), and diagonal matrix random effects (covariates: years since baseline, quadtime, participant ID), considered the

n	Healthy controls	PASS				
		Stage 0	Stage 1	Stage 2	Stage 3	
	30	15	25	19	19	
						One-Way ANOVA p value
Age [years]	56.5, 46.0–61.3	33.0, 27.0–36.0	53.0, 42.0–62.5	53.0, 43.0–64.0	65.0, 55.0–71.0	<0.0001
CAP_{100}	N/A	53.9, 47.8–66.6	85.5, 74.3–96.0	98.6, 90.6–105.1	115.9, 103.5–123.1	<0.0001
						Kruskal–Wallis or Chi Square p value
Gender n[M/F], (%)	17/13, (57/43)	7/8, (47/53)	16/9, (64/36)	11/8, (58/42)	6/13, (32/68)	0.10
Education [years]	16.0, 12.0–18.0	16.0, 13.0–18.0	16.0, 14.0–17.0	16.0, 12.0–18.0	16.0, 12.0–18.0	0.94
CAG Repeats	N/A	40.0, 40.0–42.0	41.0, 40.0–42.5	42.0, 41.0–43.0	42.0, 40.0–45.0	0.13
TMS	0.0, 0.0–0.0	0.0, 0.0–1.0	3.0, 0.5–5.5	9.0, 5.0–12.0	40.0, 28.0–53.0	<0.0001
TFC	13.0, 13.0–13.0	13.0, 13.0–13.0	13.0, 13.0–13.0	13.0, 12.0–13.0	8.0, 6.0–10.0	<0.0001
MoCA	28.0, 26.8–29.0	28.0, 27.0–29.0	27.0, 25.0–28.8	26.0, 21.8–27.5	22.0, 20.0–26.0	0.0014
SDMT	51.0, 45.0–56.3	56.0, 53.0–61.0	50.0, 44.0–53.5	34.0, 26.0–45.0	21.0, 15.0–26.0	<0.0001
cUHDRS	17.5, 16.6–17.8	17.7, 17.0–18.8	16.3, 16.1–17.5	14.3, 13.0–15.6	9.2, 7.4–10.5	<0.0001

CAP_{100}, standardised CAG-Age Product (CAP) score; cUHDRS, composite Unified Huntington's Disease Rating Scale; F, female; M, male; MoCA, Montreal Cognitive Assessment; TMS, Total Motor Score; TFC, Total Functional Capacity; SDMT, Symbol Digit Modalities Test. Healthy controls were not included in the analyses but are included in Table 4 as a qualitative indication of the general population range of values. P-values were determined using One-Way ANOVA, Kruskal–Wallis or Chi Square test as indicated, and relate to the comparison of PASS Stage 0, Stage 1, Stage 2 and Stage 3.

Table 4: **Cohort characteristics (median, interquartile range) with PASS categorisation.**

following fixed variables: intercept, years since baseline, quadtime, PASS, and [PASS]*[years since baseline].

This model gave an intercept (time-point = 0, PASS category = 0) of 29.97 pg/ml plasma NfL (95% CI = 10.58–49.36; p = 0.0028). The coefficient for PASS was β = 33.00 (95% CI = 22.67–43.32; p < 0.0001), suggesting that baseline plasma NfL values increased 33.00 pg/ml on average with each increase in PASS category. While variation in plasma NfL across time for the overall population was not significant (p = 0.16), the [PASS]*[years since baseline] coefficient was significant (β = 6.58; 95% CI = 2.076–11.076; p = 0.0047), suggesting that individuals in higher HD-ISS categories display greater increases in plasma NfL per time interval. The 'quadtime' coefficient, which represents a quadratic trajectory for plasma NfL across time, reached the threshold for significance (β = 2.94; 95% CI = 0.0042–5.87; p = 0.050). Correspondingly, the trajectory of plasma NfL with HD disease progression may be modelled by:

$$y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$

Where $\beta_0 = 29.97$, $\beta_1 = 33.00$, $\beta_2 = 6.58$, $\beta_3 = 2.94$ and X_n is the corresponding value for participant i. In other words:

Estimates plasma NfL = 29.97 + 33.00 [PASS] + 6.58 [PASS * (*Years since baseline*)] + 2.94 [(*Years since baseline*)^2]

Plasma NfL trajectories within PASS categories

As with HD-ISS categorisation, we similarly plotted longitudinal plasma NfL values by PASS category (Fig. 3) and determined the corresponding linear equations, where y = plasma NfL value, and X = the number of years since baseline.

Stage 0 Y = 3.98X + 32.80

Stage 1 Y = 5.80X + 55.43

Stage 2 Y = 9.66X + 93.48

Stage 3 Y = 22.65X + 129.30

Healthy Controls Y = 3.52X + 42.24

Baseline and final assessed year plasma NfL values for each PASS stage, as well as annualised rate of change, were estimated using the above formulae and are presented in Table 5. Fig. 3 additionally shows the pCMD trajectories for the same participants, with the tail-end pCMD values for each stage shown in Table 5. Plasma NfL levels within each PASS category were not significantly associated with any demographic or clinical measures, except for an association between plasma NfL and age in Stage 1 (r = 0.44, p = 0.028 (Spearman rho)) and SDMT in Stage 3 (r = –0.47, p = 0.044 (Spearman rho)) which did not pass Bonferroni p-value adjustment.

Discussion

In this study, we present a descriptive assessment of longitudinal plasma NfL levels in individuals with the HD mutation and healthy controls that extends beyond two time points. Plasma NfL levels are well-established to increase with age,[30] and we show that this is the case both in our cohort of individuals with the HD mutation, and healthy controls. Interestingly, the β coefficient for age decade was greater in the cohort of individuals with the HD mutation (β = 21.96, 95% CI = 13.68–30.25) compared to the HC cohort (β = 12.80, 95% CI = 6.96–18.63), suggesting that additional neurodegeneration is occurring in the cohort of individuals with the HD mutation. The substitution of HD-ISS category into the model instead of age decade resulted in a significant HD-ISS beta coefficient of 32.73 (95% CI = 19.57–45.88, p < 0.0001), with a non-significant y-intercept of 18.64 pg/ml (95% CI = –9.043–46.33, p = 0.18). These values suggest that at year 0 of Stage 0—at a point when individuals with the HD mutation do not exhibit any clinical symptoms or underlying pathophysiology—plasma NfL levels may be as high as 46.33 pg/ml, and subsequently increase approximately 32.73 pg/ml, and up to 45.88 pg/ml, per HD-ISS category. Similar values were provided when individuals with the HD mutation were grouped by PASS criteria (PASS β = 33.00, 95% CI = 22.67–43.32), albeit with a higher intercept (β = 29.97, 95% CI = 10.58–49.36, p = 0.0028) that was significant to the model. While individuals in PASS Stage 0 were not motorically or functionally impaired, they did present with slightly worse cognitive performance than those in HD-ISS Stage ≤1 (SDMT; Table 4 vs Table 1), likely due to different incorporation of SDMT scores into PIN scores and into the HD-ISS; as discussed further on, this discrepancy in cognitive performance may contribute to the higher plasma NfL y-intercept in the PASS model.

Overall, we show that plasma NfL levels increase temporally across the HD spectrum, starting from asymptomatic Stage 0 and increasing both with a quadratic function of time (HD-ISS model β = 3.15, 95% CI = 0.17–6.13; PASS model β = 2.94, 95% CI = 0.0042–5.87), and with the interaction between time and HD stage (HD-ISS model β = 7.85, 95% CI = 2.53–13.17; PASS model β = 6.58, 95% CI = 2.076–11.076). Notably, the degree to which plasma NfL levels are estimated to increase—as defined by the beta coefficients and 95% CI—does not greatly differ between the HD-ISS and PASS models, suggesting that the latter may be of use when investigating early

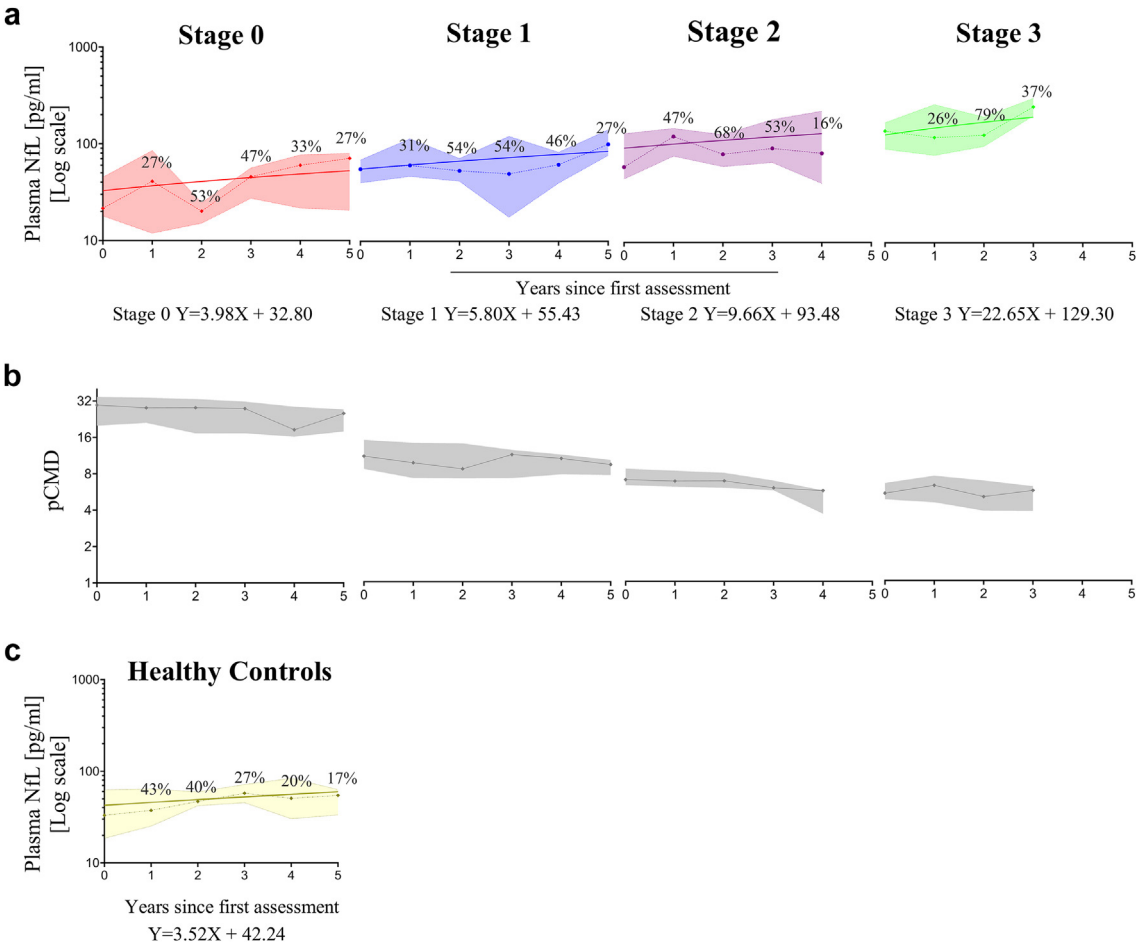

Fig. 3: A presentation of longitudinal NfL values by PASS category (Stage 0 n = 15, Stage 1 n = 25, Stage 2 n = 19, Stage 3 n = 19), determined at each participant's baseline visit (a), and the corresponding predicted years to clinical motor diagnosis (pCMD) values (b). Symbols represent the median, and shaded areas represent the interquartile range. Percentage values provided represent the percentage of participants who contributed a value at that time-point (all time 0s = 100%). In (a) and (c), the coloured linear regression line corresponds to the linear regression equation below each graph. All biological measurements conducted with two technical replicates.

neurodegeneration (i.e Stage 0 and Stage 1) in the absence of quantitative neuroimaging data.

We also investigated the change in plasma NfL levels within-stage and across time, as annualised rates and percentages of change. These findings can be compared to those reported previously by Rodrigues and colleagues, who assessed plasma NfL at two separate time-points.[21] Rodrigues reported a 2-year rate of change of 0.28 pg/ml/year for healthy controls, whereas we observed a higher rate of change of 3.52 pg/ml/year for our healthy control group. For individuals with the HD mutation, Rodrigues reported 0.84 pg/ml/year for pre-manifest HD (approximating Stage 0–2; all TFC = 13), and 1.04 pg/ml/year for manifest HD; the rate of change in our cohort of individuals with the HD mutation were well above this level, starting at 5.28 pg/ml/year for HD-ISS Stage ≤1 (or 3.82 pg/ml/year for PASS Stage 0) and reaching 18.41 pg/ml/year for HD-ISS Stage 3 (22.65 pg/ml/year for PASS Stage 3). It is unlikely that these differences in study findings are due to differences in cohort characteristics; for example, the Stage 3 cohort in our study was comparable to the manifest HD cohort in Rodrigues' study, when comparing mean age, CAG, TMS, TFC, SDMT or cUHDRS (ps > 0.05 using unpaired t-test; data not shown). Also, it is worth noting that the relative ratios for rate of change, between healthy controls, pre-manifest and HD stages, are quite similar for our study and that of Rodrigues. Therefore, instead, it is likely that the use of different NfL assay kits, from different manufacturers, has contributed to the variation between studies.[31] This is a significant limitation for the advancement of plasma NfL as an analytical and clinical biomarker of neurodegenerative disorders that has been acknowledged and is actively being investigated by the research community. It is also important to note that

Category	Year	Estimated plasma NfL level (pg/ml)	pCMD	Annual ΔPlasma NfL (pg/ml)	Annual ΔPlasma NfL (%)
Healthy Controls	Year 0	42.24	N/A	3.52	8.32
	Year 5	59.84	N/A		
PASS					
Stage 0	Year 0	32.80	28.12	3.98	12.13
	Year 5	52.70	22.87		
Stage 1	Year 0	55.43	11.93	5.80	10.46
	Year 5	84.43	8.94		
Stage 2	Year 0	93.48	7.73	9.66	10.33
	Year 4	132.12	6.01		
Stage 3	Year 0	129.30	5.70	22.65	17.52
	Year 3	197.25	5.10		

Values were calculated based on the plasma NfL, and predicted years to clinical motor diagnosis (pCMD), trajectories presented in Fig. 3.

Table 5: **Tail-end median plasma NfL and predicted years to clinical motor diagnosis values, for PASS categories and healthy controls.**

Rodrigues and colleagues 'staggered' their participants by baseline age, whereas we accounted for age by grouping participants by HD-ISS category. Reassuringly, the plasma NfL levels for our control cohort are similar to those reported for control groups of other studies,[32–35] and therefore our findings may be compared to other such studies.

Regarding the progression of plasma NfL levels in individuals with the HD mutation over time, these levels increased above that of healthy controls—who reached a peak of 59.84 pg/ml plasma NfL at year 5—within Stage 1. Significantly, as our healthy control cohort represented the 'normal' full range of plasma NfL values irrespective of age, our findings suggest that the observed Stage 1 increase is not an age-related effect—indeed, our Stage 1 cohort median age was 9.5 years younger than our median healthy control age.

In other populations (amyotrophic lateral sclerosis, frontotemporal dementia, corticobasal syndrome/progressive supranuclear palsy, amongst others), plasma NfL concentration cut-points that distinguished these populations from cognitively normal controls have previously been reported to be 38.0 pg/ml at 95% CI and 50.0 pg/ml at 99% CI.[34] The authors of this latter study note that plasma NfL showed higher accuracy in identifying neurodegenerative disorders from controls in individuals <65 years (AUC = 0.90, 95% CI = 0.86–0.93), compared to in individuals >65 years (AUC = 0.83, 95% CI = 0.82–0.86),[34] suggesting that age-related neurodegeneration may confound plasma NfL specificity in older individuals. Similarly, a study working to establish age-specific reference ranges for plasma NfL in healthy individuals determined different upper-limit reference cut-points for individuals aged 18–< 51 (10 pg/ml), those aged 51–< 61 (15 pg/ml), those aged 61–< 70 (20 pg/ml) and those above 70 years of age (35 pg/ml),[36] suggesting an increase in the confound of age from the 6th decade onwards. While the values in this latter study are below those reported in our study, this reference range study was conducted using a Simoa platform,[36] and as we have previously acknowledged, variation associated with the use of different NfL assay kits from different manufacturers and platforms remains a significant limitation in the field.[31]

Acknowledging this potential age confound, and using the data available to us in the current study, we conducted a preliminary, qualitative comparison of HD-ISS Stage 1 participants compared to HC participants who fell within the HD-ISS Stage 1 age interquartile range (35.0–62.5 years) (Supplementary Figure S3)—an approximation of age-matching. Eight controls were excluded to accomplish this age-matching. This preliminary analysis produced an estimated plasma NfL value for year 5 of the HC cohort of 54.60 pg/ml (Supplementary Table S5), further suggesting an approximate point at which individuals with the HD mutation may begin to differ from controls, although this might differ slightly depending on the platform by which NfL was measured. We note that our comparison is preliminary and qualitative, and stress that additional research should be conducted on this topic.

As we have previously surmised,[11] this increase in plasma NfL levels in Stage 1 and association with neurodegeneration cut-points is not surprising, as membership into Stage 1 using HD-ISS categorisation is governed by asymptomatic, quantitative change in putamen and caudate volume,[8] and plasma NfL levels have previously been associated with these measures.[18,19] Although quantitative neuroimaging data was not available for the current study, future research into the associations between Stage 1 landmarks (and sub-group landmarks) and plasma NfL levels, would contribute to the understanding and establishment of plasma NfL as a biomarker of early disease progression.

The use of Mixed Linear Modeling has been recommended for the longitudinal, repeated measure analysis

of neurodegenerative diseases (with a focus on HD), and is recommended by the FDA for observational studies.[37] However, it is also possible that the use of linear and quadratic terms in our mixed linear model, and use of linear equations for our within-HD-ISS models, limit the full range of potential models being considered. Therefore, we also conducted a fractional polynomial regression analysis, in order to consider additional models. While this analysis could not consider HD-ISS category or repeated measures in the same way, and therefore produced a different model equation, estimated plasma NfL levels at the tail-ends of each stage, as predicted using the fractional polynomial regression analysis, and our within-HD-ISS linear plot analyses, were similar (Supplementary Table S6). Therefore, it is possible that multiple models and equations exist for the same data, depending on the variable under investigation. For example, our HD-ISS linear equations would be preferrable when the HD-ISS category is known, and the degree of progression through that category based on plasma NfL levels is desired. Conversely, the fractional polynomial equation would be preferrable, when an individual's plasma NfL level is known, and their estimated HD-ISS category is desired in the absence of quantitative neuroimaging data and neurological assessment. Our mixed linear model approach was used to understand the plasma NfL trajectory across time, and—as the equation produced requires knowledge of HD-ISS category and years since baseline or first assessment within a HD-ISS category, we do not anticipate that the equation will have foreseeable real-life use.

Overall, in the current study, plasma NfL levels increased semi-linearly across the earlier stages of the disease (Stage $\leq$1), during which time these levels primarily correlated with age, as well as a cognitive measure that has been shown to be very sensitive to disease progression (SDMT). These observations are in line with our previous study, where we found a significant association between plasma NfL and SDMT scores in premanifest HD participants.[9] In support of these observations, the current literature contains a plethora of evidence suggesting an association between plasma NfL levels and cognitive function, across a range of disorders. These previous findings include associations with worse scores and decline in domains of memory, language, attention and global cognition,[38] as well as decline on the Stroop test[39] in dementia-free, older adults; however, in other instances, associations with domain and global cognition scores were not found.[40] Since we also did not detect an association between plasma NfL and global cognition, as measured by the MoCA, it is possible that plasma NfL levels are more sensitive to either subclinical or early cognitive decline—prior to any noticeable differences on global measures such as the MocA—or a specific aspect of cognitive decline, such as processing speed.[41]

In the current study, we did not observe any significant correlations with HD-ISS Stage 2 that passed Bonferroni p-value correction; however, the small Stage 2 sample size may have affected this result. For HD-ISS Stage 3, plasma NfL levels were also correlated with TMS. This correlation between plasma NfL levels and TMS was not observed in our previous publication[9]; it is likely that this discrepancy is associated with our grouping of participants into HD-ISS categories (i.e. Stage 2 vs. 3) in the current study, versus our grouping of participants into premanifest vs. manifest HD in our previous study. Manifest HD is clinically determined at the onset of (or first research or clinic visit following) motor symptoms unequivocally associated with HD, whereas HD-ISS Stage 3 is determined by functional impairment that follows a research-determined threshold of cognitive or motor impairment. Therefore, plasma NfL may be associated with overall disease progression, as evidenced by Figs. 1 and 3 or, specific to Stage 3, motor dysfunction in the presence of functional impairment. In support of this hypothesis, we also noted a significant correlation between plasma NfL and the cUHDRS (which encompasses motor (TMS), functional (TFC), and cognitive (SDMT, SWR) scales) in HD-ISS Stage 3, also not reported in our previous study. Preliminary, qualitative observations (Figs. 1 and 3), coupled with calculated increases in plasma NfL rate of change (Tables 2 and 5) suggest an elevation in annualised plasma NfL levels in Stage 3. Such increases may suggest a transition from prodromal to manifest HD and subsequent increase in motorically driven functional impairment. We acknowledge that limited sample sizes at these later time-points of Stage 3 prevent a comprehensive investigation of this phenomenon in the current study, but highly recommend future research into this possibility. Notably, no significant correlations were observed when participants were categorised by PASS. While it may be that the smaller sample sizes reduced our power to detect such correlations in these cohorts, it is also possible that by categorising participants by PIN scores, which encompass SDMT and TMS, we have already corrected for these factors.

There are some limitations associated with this study. First, there is no specific length of time for which a participant will remain in a particular stage, and therefore some participants may have transitioned to the next stage before the 5 years depicted in Figs. 1 and 3. Future studies powered to investigate the longitudinal association between plasma NfL levels and stage conversion or transition would be beneficial. In addition, as participants were placed in 'baseline' (time 0) stages predicated on their first study appointment at UCSD HDSA CoE, it is possible that some such participants were mid-way through that stage at the time, which would contribute to the observed variation in plasma NfL levels. Next, we acknowledge the relatively small sample size included in each stage, limited by the inclusion of only participants who provided two or more longitudinal samples. Finally, the absence of age-

matched control groups for each HD-ISS stage limits our ability to compare plasma NfL levels across HD disease progression to that of controls at the same point in life. In the current study, we opted to exclude our HC group from statistical comparisons (e.g Tables 1 and 4), and limit inclusion to qualitative comparisons (e.g Table 2, Supplementary Figure S3, Supplementary Table S5, and presented separately in Figs. 1 and 3). We acknowledge that direct comparison between HD-ISS categories and controls should be conducted with a reasonable degree of caution in this study. Overall, replication of this study with a larger sample size from a different study site or cohort would be beneficial to the advancement of plasma NfL as a biomarker of HD.

Taken together, we present a singular investigation of plasma NfL values across more than 2 visits in a large cohort of well-characterised individuals with the HD mutation. Our longitudinal analysis of plasma NfL levels within the context of the newly proposed HD-ISS offers insight into the progression of the disease that contributes significantly to our understanding of the underlying neurodegenerative pathophysiology of HD. Future research into the association between plasma NfL levels and both cognitive and motoric decline, including the utility of plasma NfL in predicting this decline, would be beneficial to the HD research community. We also stress that the next obligatory step in the analytical validation of plasma NfL as a biomarker in neurodegenerative disease would be the standardisation of assays by manufacturers and researchers; such investigations are currently underway.

Contributors

Conceptualisation, E.A.T and J.C-B; Formal analysis, G.M.P; Funding acquisition, E.A.T and J.C-B; Supervision, E.A.T and J.C-B; Writing—original draft, G.M.P; Writing—review & editing, E.A.T and J.C-B. All authors have directly acessed and verified the underlying data reported in the manuscript. All authors have read and approved the final version of the manuscript.

Data sharing statement

Anonymised summary data are available from the corresponding author, following publication, by reasonable formal request from qualified researchers, subject to a signed data sharing agreement and in compliance with the requirements of the funding bodies and institutions.

Declaration of interests

J.C-B has participated in Speakers Bureaus for Teva Pharmaceuticals and EMD Serono; J.C-B has participated on a Data Safety Monitoring Board for UniQure. The authors have no other conflicts of interests to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105173.

References

1 Goh AM, Wibawa P, Loi SM, Walterfang M, Velakoulis D, Looi JC. Huntington's disease: neuropsychiatric manifestations of Huntington's disease. *Australas Psychiatr*. 2018;26(4):366–375.
2 Siesling S, Van Vugt JP, Zwinderman KA, Kieburtz K, Roos RA. Unified Huntington's disease rating scale: a follow up. *Mov Disord*. 1998;13(6):915–919.
3 Long JD, Langbehn DR, Tabrizi SJ, et al. Validation of a prognostic index for Huntington's disease. *Mov Disord*. 2017;32(2):256–263.
4 Penney Jr JB, Vonsattel JP, Macdonald ME, Gusella JF, Myers RH. CAG repeat number governs the development rate of pathology in Huntington's disease. *Ann Neurol*. 1997;41(5):689–692.
5 Warner JH, Long JD, Mills JA, et al. Standardizing the CAP score in Huntington's disease by predicting age-at-onset. *J Huntingtons Dis*. 2022;11(2):153–171.
6 Zhang Y, Long JD, Mills JA, et al. Indexing disease progression at study entry with individuals at-risk for Huntington disease. *Am J Med Genet B Neuropsychiatr Genet*. 2011;156(7):751–763.
7 Langbehn DR, Hayden MR, Paulsen JS, PREDICT-HD Investigators. CAG-repeat length and the age of onset in Huntington disease (HD): a review and validation study of statistical approaches. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153(2):397–408.
8 Tabrizi SJ, Schobel S, Gantman EC, et al. A biological classification of Huntington's disease: the Integrated Staging System. *Lancet Neurol*. 2022;21(7):632–644.
9 Parkin GM, Corey-Bloom J, Snell C, Castleton J, Thomas EA. Plasma neurofilament light in Huntington's disease: a marker for disease onset, but not symptom progression. *Parkinsonism Relat Disord*. 2021;87:32–38.
10 Parkin GM, Corey-Bloom J, Long JD, Snell C, Smith H, Thomas EA. Associations between prognostic index scores and plasma neurofilament light in Huntington's disease. *Parkinsonism Relat Disord*. 2022;97.
11 Parkin GM, Thomas EA, Corey-Bloom J. Plasma NfL as a prognostic biomarker for enriching HD-ISS stage 1 categorisation: a cross-sectional study. *eBioMedicine*. 2023;93.
12 Bridel C, Van Wieringen WN, Zetterberg H, et al. Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. *JAMA Neurol*. 2019;76(9):1035–1048.
13 Gaiottino J, Norgren N, Dobson R, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. *PLoS One*. 2013;8(9):e75091.
14 Lu C-H, Macdonald-Wallis C, Gray E, et al. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. *Neurology*. 2015;84(22):2247–2257.
15 Scherling CS, Hall T, Berisha F, et al. Cerebrospinal fluid neurofilament concentration reflects disease severity in frontotemporal degeneration. *Ann Neurol*. 2014;75(1):116–126.
16 Weston PS, Poole T, Ryan NS, et al. Serum neurofilament light in familial Alzheimer disease: a marker of early neurodegeneration. *Neurology*. 2017;89(21):2167–2175.
17 Zhou W, Zhang J, Ye F, et al. Plasma neurofilament light chain levels in Alzheimer's disease. *Neurosci Lett*. 2017;650:60–64.
18 Byrne LM, Rodrigues FB, Blennow K, et al. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. *Lancet Neurol*. 2017;16(8):601–609.
19 Byrne LM, Rodrigues FB, Johnson EB, et al. Evaluation of mutant huntingtin and neurofilament proteins as potential markers in Huntington's disease. *Sci Transl Med*. 2018;10(458):eaat7108.
20 Niemelä V, Landtblom AM, Blennow K, Sundblom J. Tau or neurofilament light—which is the more suitable biomarker for Huntington's disease? *PLoS One*. 2017;12(2):e0172762.
21 Rodrigues FB, Byrne LM, Tortelli R, et al. Mutant huntingtin and neurofilament light have distinct longitudinal dynamics in Huntington's disease. *Sci Transl Med*. 2020;12(574).
22 Nasreddine ZS, Phillips NA, Bédirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53(4):695–699.
23 Smith A. *Symbol digit modalities test. The clinical neuropsychologist*. 1973.
24 MacLeod C. *The Stroop task in clinical research. Cognitive methods and their application to clinical research*. 2005:41–62.
25 Unified Huntington's disease rating scale. Reliability and consistency. Huntington Study Group. *Mov Disord*. 1996;11(2):136–142.

26 Schobel SA, Palermo G, Auinger P, et al. Motor, cognitive, and functional declines contribute to a single progressive factor in early HD. *Neurology*. 2017;89(24):2495–2502.
27 Long JD, Gantman EC, Mills JA, et al. Applying the Huntington's disease integrated staging system (HD-ISS) to observational studies. *J Huntingtons Dis*. 2023;12(1):57–69.
28 Heck RH, Thomas SL, Tabata LN. *Multilevel and longitudinal modeling with IBM SPSS*. Routledge; 2013.
29 NCSS 2024 Statistical Software. *NCSS, LLC*. Utah, USA: Kaysville; 2024. ncss.com/software/ncss.
30 Bornhorst JA, Figdore D, Campbell MR, et al. Plasma neurofilament light chain (NfL) reference interval determination in an age-stratified cognitively unimpaired cohort. *Clin Chim Acta*. 2022;535:153–156.
31 Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med*. 2016;54(10):1655–1661.
32 He L, Morley JE, Aggarwal G, Nguyen AD, Vellas B, de Souto Barreto P. Plasma neurofilament light chain is associated with cognitive decline in non-dementia older adults. *Sci Rep*. 2021;11(1):1–9.
33 Lewczuk P, Ermann N, Andreasson U, et al. Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. *Alzheimer's Res Ther*. 2018;10:1–10.
34 Ashton NJ, Janelidze S, Al Khleifat A, et al. A multicentre validation study of the diagnostic value of plasma neurofilament light. *Nat Commun*. 2021;12(1):3400.
35 Wu J, Wu D, Liang Y, Zhang Z, Zhuang L, Wang Z. Plasma neurofilament light chain: a biomarker predicting severity in patients with acute ischemic stroke. *Medicine*. 2022;101(26):e29692.
36 Simrén J, Andreasson U, Gobom J, et al. Establishment of reference values for plasma neurofilament light based on healthy individuals aged 5–90 years. *Brain Commun*. 2022;4(4):fcac174.
37 Garcia TP, Marder K. Statistical approaches to longitudinal data analysis in neurodegenerative diseases: Huntington's disease as a model. *Curr Neurol Neurosci Rep*. 2017;17(2):14.
38 Marks JD, Syrjanen JA, Graff-Radford J, et al. Comparison of plasma neurofilament light and total tau as neurodegeneration markers: associations with cognitive and neuroimaging outcomes. *Alzheimer's Res Ther*. 2021;13:1–14.
39 van Arendonk J, Wolters FJ, Neitzel J, et al. Plasma neurofilament light chain in relation to 10-year change in cognition and neuroimaging markers: a population-based study. *Geroscience*. 2024;46(1):57–70.
40 Mielke MM, Syrjanen JA, Blennow K, et al. Plasma and CSF neurofilament light: relation to longitudinal neuroimaging and cognitive measures. *Neurology*. 2019;93(3):e252–e260.
41 Travica N, Berk M, Marx W. Neurofilament light protein as a biomarker in depression and cognitive function. *Curr Opin Psychiatry*. 2022;35(1):30–37.