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Supplementary appendix

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Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis

Supplementary Appendix

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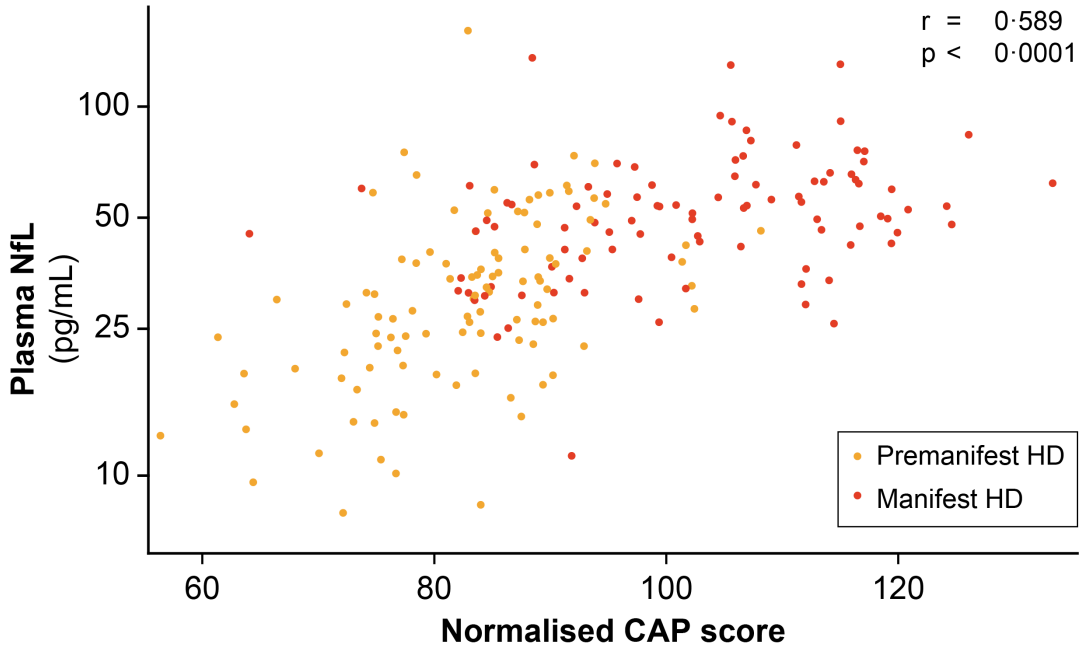
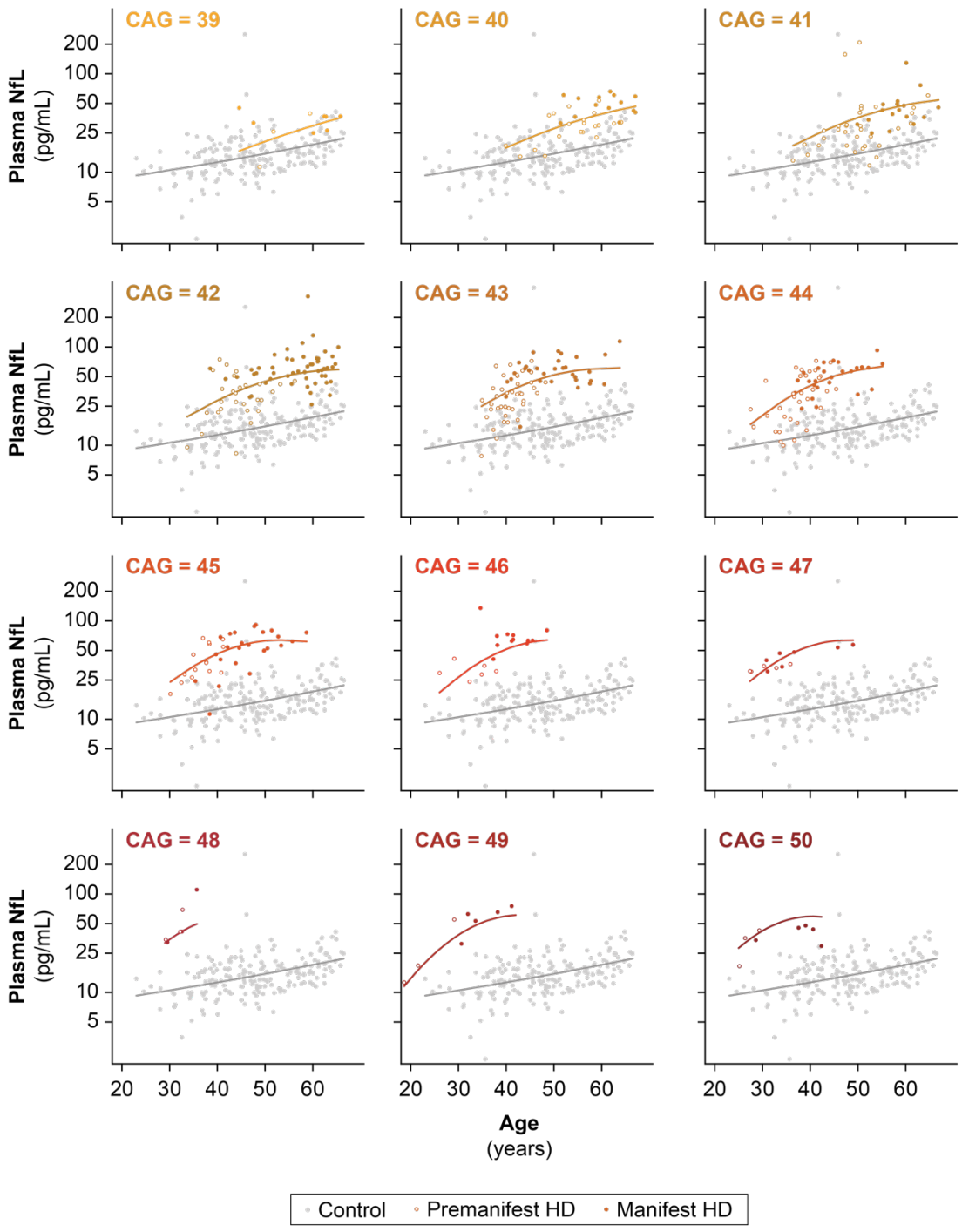
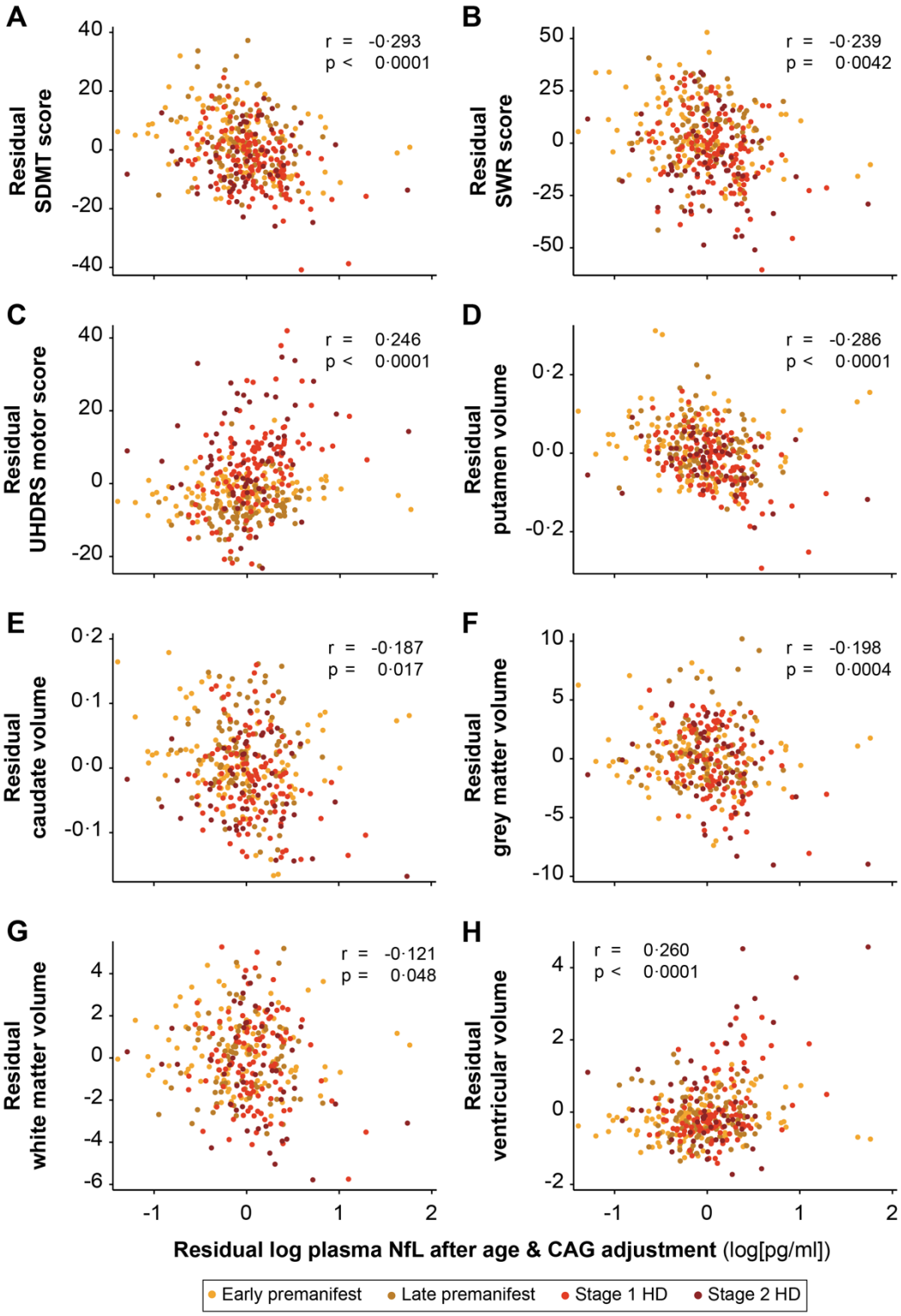


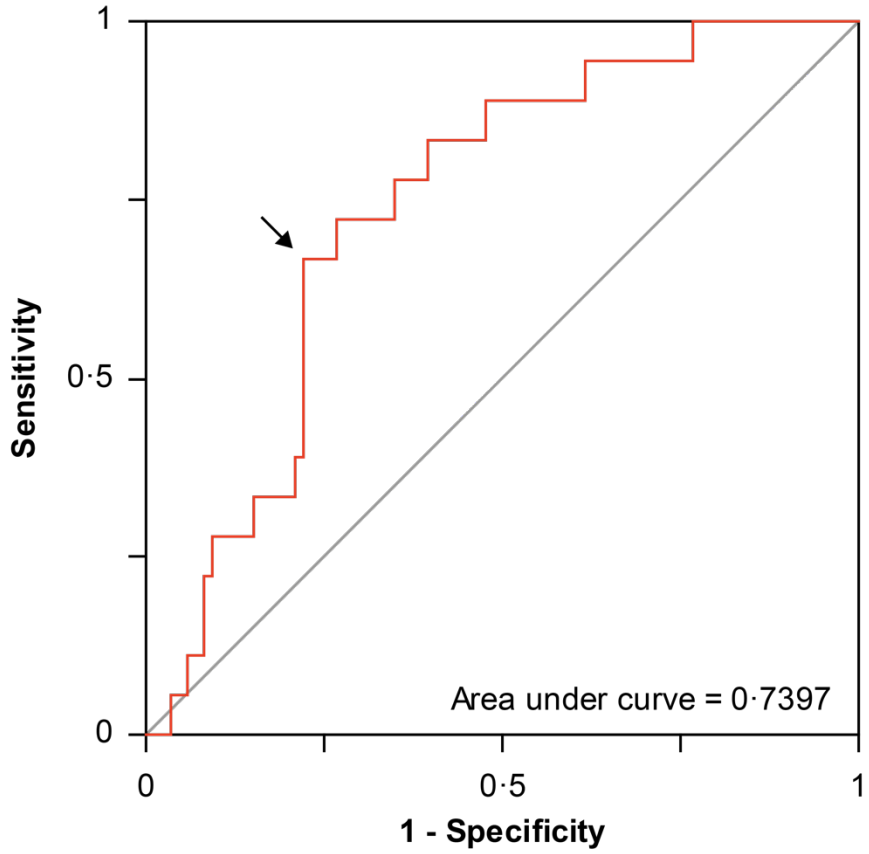
Supplementary Figure 1. Relationship between plasma NfL and normalised CAG-age product (CAP) score in HD mutation carriers. The normalised CAP score (Ross *et al.*, Nat Rev Neurol 2014; 10(4): 204-16) is an estimate of an individual's lifetime exposure to mutant huntington based on age and CAG repeat length. Given by **CAP = 100 × age × (CAG - 30) ÷ 627**, it is closely related to the 'disease burden score' of Penney *et al.* (Ann Neurol 1997; 41(5):689-92) but standardises such that a score of 100 represents the predicted age of onset from the conditional probability model of Langbehn *et al.* (Clin Genet 2004; 65(4):267-77).



Supplementary Figure 2. Relationship between plasma NfL and age for each individual CAG repeat count and controls, modelled as per Figure 1B.



Supplementary Figure 3. Relationship between cross-sectional clinical and imaging measures after adjustment for age and CAG repeat length. Both axes show the residuals from the generalized linear model incorporating significant terms from the age-CAG analysis described in **Supplementary Table 3**. Abbreviations and vertical axis units are as per Figure 2.



Supplementary Figure 4. Receiver operating characteristic (ROC) curve showing diagnosis risk within 36 months in the premanifest cohort. The point of highest mean sensitivity (0·667) and specificity (0·779), indicated by arrow, was at plasma NfL=3·61 log[pg/ml], close to the premanifest HD median value of 3·69.

Supplementary Table 1. Baseline characteristics of each cohort. A. TRACK-HD cohort. B. CSF cohort. Values are mean ± SD. CAG, CAG triplet repeat count; TMS, total motor score; TFC, total functional capacity; CAP, standardised CAG-age product score; 5yr onset prob, conditional probability of onset within 5 years (Langbehn et al, Am J Hum Genet 2009: 135B, 397-408). Disease duration is based on a rater's estimation of disease onset. Disease duration was available for 90 of the TRACK-HD manifest HD subjects. *pooled HD group includes 3 premanifest mutation carriers.

A. TRACK-HD cohort						B. CSF cohort			
Group	**Control**	**Early premanifest**	**Late premanifest**	**HD Stage 1**	**HD Stage 2**	**Control**		**HD***	
n	97	58	46	66	31	14	(13 plasma)	23	(17 plasma)
Age	46·08 ± 9·91	41·22 ± 8·52	40·68 ± 8·94	47·70 ± 10·32	51·29 ± 8·23	43·52 ± 13·17		50·11 ± 11·28	
Sex (M:F)	41:56	26:32	22:24	27:39	17:14	04:10		10:13	
CAG	N/A	42·14 ± 1·84	44·17 ± 2·39	43·62 ± 3·37	43·55 ± 2·46	N/A		43·6 ± 2·43	
TMS	1·51 ± 1·63	2·19 ± 1·42	2·96 ± 1·89	19·33 ± 9·28	30·29 ± 9·82	N/A		28·65 ± 22·11	
TFC	12·99 ± 0·10	12·90 ± 0·41	12·76 ± 0·78	12·27 ± 0·85	8·74 ± 1·06	13 ± 0		10·04 ± 3·04	
CAP	N/A	77·62 ± 7·88	88·93 ± 6·75	99·26 ± 12·67	108·54 ± 12·41	N/A		106·15 ± 19·20	
5yr onset prob	N/A	0·14 ± 0·061	0·32 ± 0·085	0·44 ± 0·16	0·53 ± 0·15	N/A		N/A	
Disease duration	N/A	N/A	N/A	5·53 ± 6·68	8·74 ± 4·68	N/A		N/A	

Supplementary Table 2. Baseline measures in the Track-HD cohort. A. NfL, clinical and imaging measures. **B.** Relationships between plasma NfL and CAP score and 5-year conditional onset probability. **C.** Inter-group differences, confidence intervals and effect sizes for baseline plasma NfL. Effect size is least square mean difference scaled by residual standard deviation in the underlying ANOVA model. CAP score, normalised CAG-age product score.

A		Control	Early premanifest	Late premanifest	HD stage 1	HD stage 2
	n	97	58	46	66	31
	Plasma NfL	18·11 ± 25·61	28·36 ± 22·24	39·39 ± 14·19	52·18 ± 20·52	57·48 ± 23·82
	log plasma NfL	2·68 ± 0·52	3·17 ± 0·56	3·61 ± 0·37	3·89 ± 0·35	3·96 ± 0·48
	SDMT	53·54 ± 8·99	52·83 ± 9·69	49·8 ± 11·36	37·35 ± 9·14	31·00 ± 9·42
	SWR	107·07 ± 16·29	102·19 ± 15·43	96·70 ± 17·55	84·56 ± 15·01	70·19 ± 19·87
	Whole-brain	81·30 ± 3·61	80·57 ± 3·63	78·54 ± 4·41	76·19 ± 4·57	71·69 ± 3·94
	Caudate	0·55 ± 0·058	0·48 ± 0·066	0·45 ± 0·073	0·38 ± 0·076	0·33 ± 0·06
	Putamen	0·70 ± 0·075	0·62 ± 0·10	7·42 ± 1·15	0·46 ± 0·93	0·43 ± 0·055
	Grey matter	46·36 ± 3·61	46·1 ± 2·92	669·29 ± 77·01	42·98 ± 3·39	40·69 ± 3·74
	White matter	32·94 ± 1·88	32·47 ± 1·53	31·08 ± 1·83	30·14 ± 2·14	28·78 ± 2·22
	Ventricles	1·12 ± 0·65	1·11 ± 0·53	1·26 ± 0·59	1·77 ± 0·84	2·36 ± 1·25

B		n	Pearson r	p-value
	CAP score	201	0·589	<0·0001
	5yr onset prob	201	0·589	<0·0001

C	Group 1	Group 2	Difference Between Means	95% Confidence Limits		Effect Size	P-value
	Control	**Early premanifest**	-0·49	-0·644	-0·336	2·21	<0·0001
	Control	**Late premanifest**	-0·928	-1·094	-0·762	4·18	<0·0001
	Control	**HD stage1**	-1·213	-1·36	-1·065	5·46	<0·0001
	Control	**HD stage2**	-1·276	-1·467	-1·084	5·75	<0·0001
	Early premanifest	**Late premanifest**	-0·438	-0·621	-0·255	1·97	<0·0001
	Early premanifest	**HD stage1**	-0·722	-0·889	-0·556	3·25	<0·0001
	Early premanifest	**HD stage2**	-0·785	-0·992	-0·579	3·54	<0·0001
	Late premanifest	**HD stage1**	-0·285	-0·463	-0·106	1·28	<0·0001
	Late premanifest	**HD stage2**	-0·348	-0·563	-0·132	1·57	0·0007
	HD stage1	**HD stage2**	-0·063	-0·265	0·139	0·28	0·5115

Supplementary Table 3 Relationships between plasma NfL, age and CAG repeat. A. Relationship between plasma NfL (log), age and *HTT* CAG repeat count in HD mutation carriers, examined using a polynomial function allowing for interactions with these predictors, their squares and all potential interactions. Age was centred at 50 years; CAG count at 42. DF, 198 degrees of freedom. **B.** Relationship between plasma NfL (log) and age in controls, 96 residual degrees of freedom. The relationship is essentially linear. (The nonsignificant Age^2 term is retained for consistency with the above model for participants with *HTT* CAG expansion.)

A

Effect	Estimate	Standard Error	t Value	P value
Intercept	3·8007	0·03814	99·65	<.0001
Age	0·03408	0·003735	9·12	<.0001
CAG	0·1866	0·02227	8·38	<.0001
Age × CAG	-0·00456	0·002469	-1·85	0·0661
Age^2	-0·00106	0·00036	-2·95	0·0036
CAG^2	-0·02331	0·006174	-3·78	0·0002
Age^2 × CAG	-0·00026	0·000105	-2·47	0·0143
Age × CAG^2	-0·00091	0·000279	-3·24	0·0014

B

Effect	Estimate	Standard Error	t Value	P value
Intercept	2·7463	0·05044	54·45	<.0001
Age	0·02061	0·004162	4·95	<.0001
Age^2	0·000066	0·000333	0·2	0·8435

Supplementary Table 4. A. Further detail of survival analysis in premanifest cohort. **B. Tests of equality in the survival analysis using log-rank and Wilcoxon tests.** P values given are asymptotic approximations. An exact permutation method for real-valued log-rank scores (exactRankTests v0.8-29 package for R v3.3.3), gave a 2-sided p value of 0.00247. **C. Log hazard ratio for the prediction of new diagnosis as a function of log plasma NfL concentrations after controlling for other known predictors.** Analysis was done in the group that was premanifest at baseline. Due to the limited number of new diagnoses (18 over 36 months), covariate adjustments were controlled one at a time, except for age, CAG length, and their interaction. The log NfL hazard ratio is little changed by additional of any of these covariates, suggesting that NfL is a risk predictor that is not redundant with other known predictors. [(a)]For context, the standard deviation of log NfL concentration in at-risk preHD subjects was 0·39 log(pg/ml) (See **Supplementary Table 2**).

A

Time (yrs)	Survival	Failure	Survival SE	Failed	Remaining	95% CI	
NfL above median							
0	1.000	0.000	0	0	52	_	_
1	0.923	0.077	0.037	4	48	0.851	0.996
2	0.827	0.173	0.0525	9	43	0.724	0.930
3	0.712	0.289	0.0628	15	37	0.588	0.835
NfL below median							
Time (yrs)	**Survival**	**Failure**	**Survival SE**	**Failed**	**Remaining**	**95% CI**	
0	1.000	0.000	0	0	52	_	_
1	0.981	0.019	0.019	1	51	0.944	0.999
3	0.942	0.058	0.0323	3	49	0.879	0.999

B

Test	Chi-Square	P value
Log-Rank	9.720	0.0018
Wilcoxon	9.721	0.0018

C

Covariate controlled	NfL log Hazard Ratio per log(pg/ml)[(a)]	Standard error	p-value
None	1·192	0·409	0·0036
Age, CAG, Age × CAG	1·109	0·532	0·0371
Caudate volume	1·084	0·451	0·0162
Putamen volume	1·213	0·548	0·0269
Whole-brain volume	1·060	0·436	0·0151
Ventricular volume	1·119	0·435	0·0101
White-matter volume	1·052	0·453	0·0201
Grey-matter volume	1·092	0·455	0·0163
UHDRS Total Motor Score	0·866	0·451	0·0549
Speeded tapping mean inter-tap time (nondominant hand)	1·318	0·406	0·0011
Symbol-digit modality test	0·923	0·449	0·0398
Indirect circle tracing time	1·512	0·471	0·0013
Paced tapping at 3 Hz (inverse standard deviation)	0·990	0·472	0·0358
Spot-the-Change	1·152	0·445	0·0096

Supplementary Table 5. The prognostic value of baseline NfL for longitudinal brain volume change in premanifest and manifest HD. After examining longitudinal predictive power of baseline plasma NfL for longitudinal brain volume change measures across all mutation carriers, it was re-examined controlling for group (premanifest or manifest HD) to examine whether plasma NfL offers additional prognostic power beyond that of group status. Where these analyses were significant, we then examined for the interaction between premanifest and manifest HD to determine whether there was a significantly different relationship with NfL between the two groups. Such relationships were identified for whole-brain and grey matter atrophy and lateral ventrical expansion, all of which were more strongly predicted by NfL in the manifest HD group. Regression estimate represents the least square mean slope for preHD and early HD. Unit for 'estimate' is percent TIV change per log(pg/ml) plasma NfL. * represents the group with the signifcantly stronger NfL association. For context, the standard deviation of log NfL concentration in preHD subjects was 0·39 log(pg/ml) and in early HD was 0·52log(pg/ml) (see **Supplementary Table 2**).

Longitudinal measure	Adjusting for age, CAG and group		PreHD vs Early HD Interaction					
	Regression estimate	p-value	PreHD slope estimate	p-value	Early HD slope estimate	p-value	Estimate difference	p-value
Putamen	0·00039	0·8161	0·00016	0·928	0·00061	0·816	0·00045	0·884
Caudate	0·00134	0·0279	0·00177	0·008	0·00091	0·343	0·00086	0·436
Whole brain	0·205	<0·0001	0·129	0·0007	0·282*	<0·0001	0·153	0·043
White matter	0·08	<0·0001	0·048	0·004	0·112	0·002	0·064	0·082
Grey Matter	0·077	<0·0001	0·011	0·496	0·144*	<0·0001	0·143	< 0·0001
Lateral Ventricle	-0·0606	<0·0001	-0·024	0·004	-0·097*	<0·0001	0·073	0·002

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