

Comparative analysis of neurofilament light chain in Huntington's disease like 2 and Huntington's disease

Journal of Huntington's Disease
2025, Vol. 14(1) 103–108
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DOI: 10.1177/18796397241300141
journals.sagepub.com/home/hun



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Abstract

Huntington's disease-like 2 (HDL2) closely resembles Huntington's disease (HD) in clinical and pathological features. Neurofilament light chain (NfL) is an important biomarker in HD research and holds potential in HDL2. To evaluate NfL's utility in HDL2, a comparative analysis among HDL2 (n = 12), HD (n = 9), and unaffected controls (n = 9) was conducted. Employing a cross-sectional design, NfL levels were assessed in blood plasma. Concentrations were notably elevated in both HD and HDL2 groups compared to controls. HD patients displayed higher NfL levels than HDL2, possibly reflecting disease duration differences. NfL effectively distinguished HDL2 from controls, highlighting its promise as a possible biomarker in HDL2 research.

Keywords

Huntington's disease, Huntington's disease like 2, HDL2, neurofilament light chain, biomarkers, Huntington's disease phenocopies, South Africa

Received: 30 July 2024; accepted: 30 October 2024

Introduction

Huntington's disease like-2 (HDL2) is a neurodegenerative disorder associated with a CTG/CAG triplet repeat mutation at the junctophilin 3 (JPH3) gene on chromosome 16.¹ The clinical phenotype and autosomal dominant inheritance pattern categorize HDL2 as one of the Huntington's disease (HD) phenocopies.² HDL2 shares clinical, radiological, and pathological similarities with HD, rendering them clinically indistinguishable.^{3–9} In both conditions symptoms typically emerge during productive adulthood and consist of a progressive movement disorder and dementia with variable psychiatric features⁴ resulting in a fatal outcome.³ HDL2 has been exclusively described in patients with an African ancestry and is the most common HD phenocopy in this group.^{3,9,10}

The parallels between HD and HDL2 have resulted in HDL2 studies using similar methods of investigation. These studies have supported HDL2 having a similar yet more severe trajectory.¹¹ The Unified Huntington's Disease Rating Scale (UHDRS) has been used to compare the clinical features of HDL2 with those of HD. This study showed that motor features in HDL2 had greater severity for similar disease durations which may account for patients with HDL2 presenting years earlier compared

to those with HD. Similarly, MRI brain volumetry, an important biomarker used to assess disease progression in HD,^{11,12} has reported differences in thalamic and caudate volumes not been previously found with qualitative analysis.⁵

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Neurofilament light chain (NfL) is the smallest of three subunits of the neurofilaments that compose the neuronal cytoskeleton. It is released by damaged neurons and high plasma and CSF concentrations of NfL are found in several neurological diseases.¹³ NfL is an important biofluid biomarker in HD.¹⁴ It has been shown to predict disease progression¹⁴ and is being evaluated as a biomarker of both safety and treatment efficacy in clinical trials.^{14–18}

Since NfL is a core biomarker in HD and other neurodegenerative diseases, it may have comparable value for HDL2. The objective of this study was to measure NfL in the blood plasma of patients with HDL2 and compare them to matched patients with HD and unaffected control cases.

Methods

This cross-sectional study compared clinical and genetic data, along with NfL plasma concentrations, among patients with HDL2 and HD, along with unaffected matched controls all with African ancestry. The three groups were matched for age at sampling, and the affected groups were also matched for abnormal CTG/CAG triplet repeat length. Patients diagnosed with HDL2 or HD between January 2015 and April 2018 were identified through the Division of Human Genetics, National Health Laboratory in Johannesburg and invited to enroll in this study. Controls were genetically unrelated healthy acquaintances and were frequency-matched for age. Participants with significant head injury, HIV or other known neurological disorders were excluded from the study. The UHDRS was used to assess the disease affected groups at the time that the plasma sample was taken.

Blood samples were obtained in EDTA tubes. The plasma was separated then stored and frozen at -80°C . Plasma samples were then sent to University College London HD Centre for NfL analysis.¹⁵ NfL was quantified in duplicate using the Neurology 4-Plex B assay on the Simoa HD-X Analyzer (Quanterix). All samples were analysed within the same run using the same batch of reagents. The intra-assay coefficient of variation was 4.08%.

This study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M140872).

Statistical analyses

The NfL distribution was tested for normality and a natural logarithm transformation applied to normalize the distribution. Independent samples t-test or one-way Analysis of Variance (ANOVA) and Pearson's chi-squared test were used to compare baseline characteristics of the three groups. Intergroup biomarker comparisons were made using Analysis of Covariance (ANCOVA) using log (NfL) as the dependent variable, diagnosis group as the

independent variable, and age at blood sampling, age at blood sampling and abnormal repeat length, and disease duration (time from the first reported symptom to the time of the biomarker testing) as covariates. As a sensitivity analysis, the NfL analyses were repeated using a non-parametric ANCOVA.

To understand the NfL plasma level differences between groups (i.e., intergroup discrimination) Receiver Operating Characteristic (ROC) curves with age at blood sampling as covariate were generated. Correlations between NfL and clinical variables were assessed using Spearman's correlation with adjustment for partial correlations for age at sampling. Data analysis was carried out using SAS version 9.4 for Windows. A 5% significance level was used. The false discovery rate was controlled at 5% by the Benjamini-Hochberg procedure in a test-wise fashion.

Results

The sample ($n = 30$) characteristics and results are summarized in the comparative Table 1. The groups were not significantly different ($p > 0.05$) in terms of age at blood sampling, age of disease onset, age of disease diagnosis, repeat length of the CAG triplet, and UHDRS - Total Functional Capacity (TFC). The disease affected groups were significantly different with respect to size of the normal allele and the duration of disease, all with lower values for the HDL2 group.

Group comparisons of the HDL2, HD and control group found significant differences in log NfL values between the three groups ($p = 0.0006$), with the HD and HDL2 groups having higher concentrations in plasma compared to the control group as anticipated (Figure 1). Further adjustments for age and abnormal repeat length, as well as duration of disease maintained the statistical significance when comparing the groups (HDL2 vs. Control: $p = 0.013$, HD vs. HDL2: $p = 0.0082$ when adjusted for age and repeat length and $p < 0.0001$ adjusted for disease duration). No significant correlations were observed between NfL concentrations and UHDRS outcomes (TMS and TFC) when applying non-parametric comparisons and controlling for age (HDL2: $r = -0.258$, $p = 0.44$, HD: $r = 0.130$, $p = 0.70$) or when controlling for both age and abnormal triplet CAG repeat length (HDL2: $r = -0.266$, $p = 0.46$, HD: $r = 0.216$, $p = 0.55$).

The diagnostic power of NfL plasma (controlling for age at the time of sampling), as given by the Area Under the ROC curve data, demonstrated an excellent ability to differentiate HD and HDL2 from controls ($\text{AUC} = 0.988$, 95% CI = 0.953–1.000 for HD versus control; $\text{AUC} = 0.926$, 95% CI = 0.812–1.000 for HDL2 versus control, respectively). Furthermore, plasma NfL had good capacity to discriminate between HDL2 vs HD when controlling for age, when controlling for age and CAG repeat length, and when adjusting for disease duration.

Table 1. Comparison of the clinical and neurofilament light chain (NfL) characteristics between the Huntington's disease-like 2 (HDL2), Huntington's disease (HD) and unaffected control groups.

Group	HDL2	HD	Control	p
Descriptive Characteristics				
Number in group (n)	12	9	9	
Mutant triplet repeat length (Median (IQR))	46 (44–49)	47 (43–47)	-	p = 0.39
Wild type repeat length (Median (IQR))	14 (13–15)	17 (16–18)	-	p = 0.022 (HDL2 < HD)
Age at onset (y) (Mean (SD))	43.9 (10.6)	37.3 (15.2)	-	p = 0.30
Age at diagnosis (y) (Mean (SD))	47.1 (11.5)	45.4 (15.5)	-	p = 0.78
Duration of disease to diagnosis (y) (Mean (SD))	3.3 (2.1)	7.9 (3.4)	-	p = 0.0044 (HDL2 < HD)
Duration of disease to biomarker testing (y) (Mean (SD))	4.6 (2.8)	12.2 (3.6)	-	p = 0.0006 (HDL2 < HD)
UHDRS Total Motor Score (TMS) (Mean (SD))	40.4 (12.3)	53.9 (16.4)	-	p = 0.075
UHDRS Total Functional Capacity (TFC) (Mean (SD))	6.8 (3.4)	4.1 (2.1)	-	p = 0.075
Serum Biomarker Features				
Age at blood sampling (y) (Mean(SD))	48.4 (11.9)	49.6 (14.0)	51.3 (11.7)	p = 0.80
NfL (log pg/ml) (Mean(SD))	3.1 (0.4)	3.9 (0.8)	2.1 (0.8)	p = 0.0006 (Ctrl < HD, HDL2)
NfL Adjusted for age: HDL2 vs. Control				p = 0.0010 (Ctrl < HDL2)
NfL Adjusted for age: HD vs. Control				p = 0.0010 (Ctrl < HD)
NfL Adjusted for age: HDL2 vs. HD				p = 0.033 (HDL2 < HD)
NfL Adjusted for age and abnormal CAG: HDL2 vs. HD				p = 0.037 (HDL2 < HD)
NfL Adjusted for duration of disease: HDL2 vs. HD				p = 0.037 (HDL2 < HD)
NfL & UHDRS correlations between affected groups				
TMS adjusted for Age (r (p))	-0.368 (0.27)	0.189 (0.66)		
(95% CI)	(-0.793 to 0.298)	(-0.595 to 0.789)		
TMS adjusted for Age and CAG (r (p))	-0.310 (0.38)	0.211 (0.65)		
(95% CI)	(-0.786 to 0.397)	(-0.644 to 0.832)		
TFC adjusted for Age (r (p))	0.205 (0.55)	-0.168 (0.69)		
(95% CI)	(-0.450 to 0.717)	(-0.780 to 0.609)		
TFC adjusted for Age and abnormal CAG (r (p))	0.129 (0.72)	-0.461 (0.30)		
(95% CI)	(-0.545 to 0.702)	(-0.901 to 0.448)		
Biomarker ROC				AUC (95% CI)
Adjusted for age HDL2 vs. Control				0.926 (0.812–1.000)
Adjusted for age HD vs. Control				0.988 (0.953–1.000)
Adjusted for age HDL2 vs. HD				0.898 (0.765–1.000)
Adjusted for age and abnormal CAG: HDL2 vs. HD				0.898 (0.755–1.000)
Adjusted for duration of disease: HDL2 vs. HD				0.972 (0.917–1.000)

AUC: area under the curve; CI: confidence interval; HDL2: Huntington's disease like 2; HD: Huntington's disease; IQR: interquartile range; NfL: neurofilament light chain; SD: standard deviation; TFC: UHDRS Total Functional Capacity. TMS: UHDRS Total Motor Score; y: year. p values shown are adjusted for multiple comparisons. Bold = Significant at <0.05, Benjamini-Hochberg procedure.

Discussion

This is the first study examining NfL plasma concentration in patients with manifest HDL2 and comparing them to matched HD and control groups. The outcomes demonstrated that NfL can differentiate between either of the affected groups and the unaffected control group. NfL plasma concentration increases proportionally to the degree of axonal damage in central nervous system disorders¹⁹ including HD.^{14–17} HDL2 is a neurodegenerative disease^{20,21} and these findings likely represent axonal injury in the central nervous system of our HDL2 cohort compared to the normal control group.

Our results indicate that NfL plasma concentration was greater in the HD group compared to the HDL2 group which may be associated with the differences in disease

duration. NfL plasma concentration in HD has been shown to increase with disease duration¹⁶ and pathogenic progression.¹⁷ The pathogenesis of HD has been attributed to disruption of various cellular processes including protein misfolding, transcriptional dysregulation, mitochondrial dysfunction, and DNA damage repair which are associated with mutant huntingtin protein.²² Therefore, as our HD group's disease duration to the time of biomarker sampling was on average 7.6 years longer than the HDL2 group, a higher NfL concentration may be expected in our HD cohort.

A longer disease duration in HD is associated with greater motor disability.²³ It is notable that the HDL2 group exhibited an earlier diagnosis compared to HD, consistent with previous findings.^{4,21} Studies suggest that

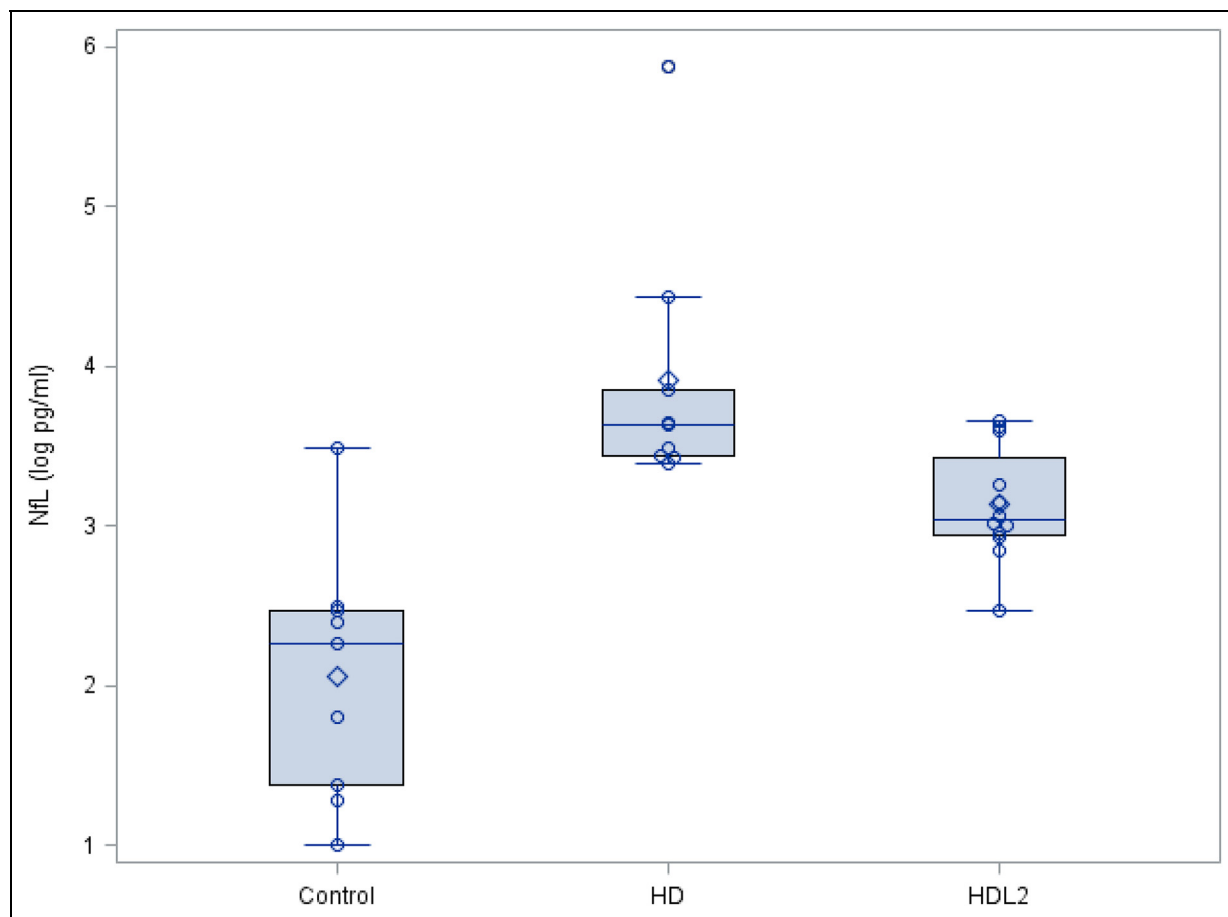


Figure 1. Box and whisker plot comparing the distributions of the Neurofilament Light chain (NfL) between Huntington's disease Like 2 (HDL2), Huntington's disease (HD) and the unaffected control groups.

Comparisons of the mean Neurofilament Light chain (NfL) plasma levels (log pg/ml) between the Huntington's disease Like 2 (HDL2) ($\bar{X} = 3.1$, $SD = 0.4$), Huntington's disease (HD) ($\bar{X} = 3.9$, $SD = 0.8$) and the unaffected control ($\bar{X} = 2.1$, $SD = 0.8$) groups found significant differences in mean log NfL values between the control and affected groups ($p = 0.0006$). Box: interquartile range (IQR) Q1-Q3 with central horizontal line representing the median. Whiskers represent the maximum and minimum values of each group. Diamonds represent the mean value of each group.

HDL2 patients may experience earlier and more severe symptoms, prompting earlier medical intervention.^{4,10} These include earlier psychiatric features⁸ and greater severity of bulbar symptoms.⁴ Despite the shorter disease duration at diagnosis, HDL2 patients show comparable UHDRS-TFC scores to HD, possibly due to the functional impact of these clinical characteristics. However, as the NfL levels are lower in HDL2 this could suggest that the HDL2 phenotype may not only be affected by the degree of neuronal damage mediated by expanded polyglutamate protein²⁴ but also influenced by other pathogenic mechanisms that differ from HD such as the loss of function of JPH3.²⁵

This first exploration of NfL in HDL2 is limited by the small sample size, which was influenced by the rarity of HDL2 and challenges with recruitment of participants which are inherent to an impoverished population. This

limits the ability to perform granular analyses of NfL trajectories by disease, identify age-specific cut offs, or examine associations between NfL and clinical sub-scores or other characteristics such as neuroimaging. NfL is clearly altered in HDL2, and plasma level trajectory may differ from that seen in HD. Further research with larger sample sizes is needed to validate these findings and establish clinical and radiological correlates with specific cut-off points for NfL concentrations in plasma.

In conclusion, NfL concentrations were significantly higher in the HD and HDL2 groups compared to the control group, and NfL concentrations between HDL2 and HD may be different even when accounting for disease duration. The findings suggest that NfL is a potentially valuable biomarker for HDL2 and NfL may have an important role in the design and conduct of clinical trials in HDL2.

Acknowledgments

The authors have no acknowledgments to report.

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Statements and declarations

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: FBR is a Medpace UK Ltd employee and was a University College London full-time employee during the conduct of this study. LMB is funded by the Medical research council (MR/W026686/1). They currently hold consultancy contracts with Annexon Biosciences, Remix Therapeutics, and LoQus23 Therapeutics Ltd via UCL Consultants Ltd. EJW reports grants from CHDI Foundation and European Huntington's Disease Network and consultancy / advisory board memberships with Annexon, Remix Therapeutics, Hoffman La Roche Ltd, Ionis Pharmaceuticals, PTC Therapeutics, Takeda, Teitur Trophics, Triplet Therapeutics and Vico Therapeutics. All honoraria for these consultancies were paid through the offices of UCL Consultants Ltd, a wholly owned subsidiary of University College London. The remaining authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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