Cross-Sectional and Longitudinal Validation of Serum Neurofilament Light Chain (NfL) as a Biomarker of Parkinson's Disease Progression

Brit Mollenhauer[1,2†], MD; Mohammed Dakna[1], PhD; Tzu-Ying Liu[23], PhD; Douglas Galasko[3], MD; Tatiana Foroud[4], PhD; Henrik Zetterberg[5,6,7,8], MD; Sebastian Schade[1], MD; Roland Gera[9], MSc Wenting Wang[23], PhD, Feng Gao[23], PhD; Niels Kruse[10], PhD; Mark Frasier[11], PhD; Lana M. Chahine[12], MD; Christopher S. Coffey,[13] PhD; Andrew B. Singleton,[14] PhD; Tanya Simuni,[15] MD; Daniel Weintraub,[16] MD; John Seibyl,[17] MD; Arthur W. Toga,[18] PhD; Caroline M. Tanner,[19] MD, PhD; Karl Kieburtz,[20] MD; Kenneth Marek[11,17], MD; Andrew Siderowf[16], MD; Jesse M. Cedarbaum[21] MD; Samantha J. Hutten[11], PhD; Claudia Trenkwalder[2*], MD; Danielle Graham[22*], PhD

*both last authors contributed equally

[1] Department of Neurology, University Medical Center Goettingen, Germany: brit.mollenhauer@med.uni-goettingen.de; mohammed.dakna@med.uni-goettingen.de; sebastian.schade@med.uni-goettingen.de
[2] Paracelsus-Elena Klinik, Kassel, Germany, ctrenkwalder@gmx.de
[3] Department of Neurosciences, University of California, San Diego, San Diego, CA: dgalasko@ucsd.edu
[4] Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA; tforoud@iu.edu
[5] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Gothenburg, Sweden.
[6] Department of Psychiatry and Neurochemistry, Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden. henrik.zetterberg@clinchem.gu.se
[7] Department of Neurodegenerative Disease, UCL Institute of Neurology, London, United Kingdom, Sweden.
[8] UK Dementia Research Institute at UCL, London, United Kingdom
[9] Department of Medical Statistics, University Medical Center Goettingen, Goettingen, Germany; roland.gera@med.uni-goettingen.de
[10] Department of Neuropathology, University Medical Center Goettingen, Goettingen, Germany; n.kruse@med.uni-goettingen.de
[11] The Michael J. Fox Foundation for Parkinson's Research, New York, NY, USA; mfrasier@michaeljfox.org; shutten@michaeljfox.org
[12] Department of Neurology, University of Pittsburgh, Pittsburgh, PA, USA; lanachahine@pitt.edu
[13]Department of Biostatistics, College of Public Health, University of Iowa, Iowa City, IA, USA: christopher-coffey@uiowa.edu
[14]Molecular Genetics Section, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA: Singleta@mail.nih.gov
[15] Parkinson's Disease and Movement Disorders Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA: Tatyana.Simuni@nm.org
[16]Department of Neurology Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA: andrew.siderowf@uphs.upenn.edu; daniel.weintraub@uphs.upenn.edu
[17]Institute for Neurodegenerative Disorders, New Haven, CT, USA: jseibyl@mnimaging.com; kmarek@mnimaging.com

[18] Laboratory of Neuro Imaging, University of Southern California (USC) Stevens Neuroimaging and Informatics Institute, Keck School of Medicine of University of Southern California, Los Angeles, CA: toga@loni.usc.edu
[19] Department of Neurology, University of California San Francisco, San Francisco, CA, USA and Parkinson's Disease Research Education and Clinical Center, San Francisco Veterans Affairs Health Care System: caroline.tanner@ucsf.edu
[20]Clinical Trials Coordination Center, University of Rochester Medical Center, Rochester, NY, USA: karl.kieburtz@ctcc.rochester.edu
[21] Coeruleus Clinical Sciences LLC, Woodbridge CT, USA; jcedarbaum@aol.com
[22] Discovery and Early Development Biomarkers, Biogen, Cambridge MA, USA; danielle.graham@biogen.com
[23] Biostatistics, Biogen, Cambridge MA, USA; wenting.wang@biogen.com; tzuying.liu@biogen.com; feng.gao@biogen.com

†Corresponding author: Brit Mollenhauer, MD
Paracelsus-Elena-Klinik and University
Medical Center, Göttingen, Germany
Klinikstrasse 16
34118 Kassel, Germany
E-Mail: brit.mollenhauer@med.uni-goettingen.de
Tel.: +49 561-6009-200

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Abstract

Background: Alterations in neurofilament light chain (NfL), reflecting axonal damage, have been proposed as a biomarker for neurological disorders including Parkinson's disease (PD).

Methods: We measured NfL concentrations by immunoassay in (1) a set of longitudinal CSF samples from 82 PD, 14 other neurodegenerative disorders (OND), and 53 healthy controls (HC); (2) A cross-sectional cohort with paired CSF and serum samples from subjects with 151 PD, 344 OND, and 20 HC, and (3a) a large longitudinal validation cohort with serum samples from 375 PD, 178 HC, and 57 prodromal Lewy Body disorder with hyposmia or isolated REM sleep behaviour disorder (iRBD), (3b) 216 symptomatic and 298 asymptomatic *LRRK2*, *GBA*, and *SNCA* mutation carriers in the Parkinson's Progression Markers Initiative (PPMI). Mean differences in serum NfL levels between diagnostic groups, and correlation with motor signs, cognitive measures and dopamine transporter imaging, were assessed. Linear mixed effects models were used to assess longitudinal changes in log10 (NfL) in relation to demographic, clinical, and imaging variables.

Findings: In the longitudinal cohort (1) NfL in CSF increased over time and was significantly positively correlated with MDS-UPDRS III motor and total scores in the PD group (p=0·0231 and 0·0081). In the cross-sectional cohort (2) the paired CSF and serum NfL samples were highly correlated (Spearman's rank $\hat{\rho} = 0 \cdot 73$; p<10^-6). In the large validation cohort (3a) mean baseline serum NfL was higher in PD (13 ±7·2pg/ml), hyposmics (15±15·1pg/ml), and iRBD (17±8pg/ml) compared with HC (12±6·7pg/ml) but was highest in the seven OND cases (18±7pg/ml). In the genetic cohort (3b), serum NfL levels were lower in asymptomatic than in symptomatic mutation carriers. There was a significant (age-adjusted) longitudinal increase in serum NfL in PD compared with HC. Serum NfL values were significantly positively associated with longitudinal MDS-UPDRS motor and total scores, as well as age.

Interpretation: We identified NfL as the first blood-based PD progression biomarker. NfL levels in serum samples are increased in PD compared to HC, increase significantly over time in PD, and correlate with a clinical measure of disease severity. Although the specificity of NfL in PD is low and additional, more specific biomarkers are needed, serum NfL is the first blood-based biomarker candidate that could support disease stratification (PD vs. OND), track clinical progression, and might be used to assess responsiveness to neuroprotective treatments.

Introduction

Two major obstacles hamper the success of translational Parkinson's disease (PD) research: 1) currently there is no longitudinal fluid biomarker for PD that correlates with clinical disease progression; 2) a definite diagnosis of PD can currently only be made by autopsy and the rate of clinical misdiagnoses, especially in the early stages of the disease, is reported to be high[1, 2]. We and others identified a 10-15% decrease in cerebrospinal fluid (CSF) α-synuclein in several related disorders including PD, multiple system atrophy (MSA), and dementia with Lewy bodies (DLB).[3, 4] CSF α-synuclein values show substantial overlap, no significant longitudinal change during 36 months follow-up, and no correlation with the progression of clinical signs and symptoms[5], limiting its clinical utility as a standalone biomarker. Therefore, additional biomarkers beyond α-synuclein are warranted that could be used for diagnosis and as progression markers in PD.

Neurofilaments are highly phosphorylated neuronal cytoskeleton components composed of three subunits of differing molecular weight maintaining neuronal structure and determining axonal calibre. The 68 kDa neurofilament light chain (NfL) is essential for the assembly of the complex as it forms its backbone and is released into extracellular fluids in response to axonal damage.[6, 7] Several studies have shown elevated levels of NfL in the CSF of patients suffering from neurodegenerative conditions.[7-9] Further, CSF NfL levels seem to reflect progression of various neurological conditions, including multiple sclerosis and neurodegenerative dementia disorders.[10, 11] Ultrasensitive methods have enabled the quantification of NfL in serum. A tight correlation between NfL in CSF and blood has emerged, thereby raising the possibility that NfL could be a blood-based biomarker for neurological disorders. NfL levels have been shown to differentiate sporadic PD from other movement disorders, such as MSA and progressive supranuclear palsy (PSP).[7, 12, 13] While one small study did not show a longitudinal change in

NfL levels during progression in PD,[14] longitudinal analyses of NfL in larger multicentre cohorts, including prodromal and monogenetic subjects, have not been carried out.

In this paper, we aim to answer the following questions: 1) Are there differences in baseline NfL levels and changes over time in the different patient groups, taking age and sex into consideration? 2) Does serum NfL correlate with CSF NfL? 3) Are NfL changes over time associated with clinical outcomes?

We hypothesized that serum NfL would: 1) be higher in PD (sporadic and with mutations) than in healthy controls (HC), 2) be even greater in subjects with other neurodegenerative disorders (OND), 3) increase over time with disease progression, 4) be higher in prodromal and asymptomatic subjects than controls, and 5) correlate with clinical outcome measures and/or imaging indices of progression

Methods

Study population

DeNoPa- and Kassel- training cohorts

The first training cohort from the longitudinal single-centre DeNoPa cohort[15, 16] was composed of 82 recently diagnosed *de novo* PD subjects with CSF and 53 HC for whom we had up to 72 months follow-up data. At 24-month follow-up, all subjects were reassessed by clinical history, response to levodopa, and neurological examination and 14/96 (15%) were diagnosed with a different disease, including atypical parkinsonism (see supplement). This group was separated from the PD group and designated thereafter as having other neurodegenerative disorder (OND, n=14).

The second training set included paired CSF and serum samples from the cross-sectional Kassel cohort and comprised 151 PD and 344 OND. We also added 20 randomly selected healthy control (HC) subjects from the DeNoPa cohort who were already included in the first training cohort. Diagnoses were rendered according to published criteria.

PPMI validation cohort

As described,[5,9] PPMI (Parkinson's Progression Marker Initiative) is an on-going prospective longitudinal, observational, international multicentre study that aims to identify PD biomarkers. The cohort consists of newly diagnosed, drug-naïve PD patients with 123-I Ioflupane dopamine transporter imaging (DaT) deficit and age- and sex-matched HC (http://ppmi-info.org/study-design). Inclusion- and exclusion criteria have been published elsewhere and can be found in the online supplement.[9] Prodromal participants with isolated REM sleep behaviour disorder (iRBD) or isolated hyposmia were additionally recruited by the PPMI-centres. For more information, including clinical assessment and imaging from this core group see supplement.

The PPMI genetic cohort comprised individuals with pathogenic mutations in *LRRK2*, *GBA*, and *SNCA*, and was stratified according to neurological evaluation into symptomatic and asymptomatic mutation carriers. Recruitment of the genetic cohort was performed through a variety of approaches (ppmi-info.org). Mutations included here are given in the supplement. Subjects for whom we had complete data and at least two serum samples were analysed and included: 375 PD, 178 HC (both until the 60-month follow-up visit), 35 iRBD, and 22 hyposmic participants (both until the 36-month visit). Seven subjects were initially enrolled as PD but upon clinical follow-up their diagnoses were revised to OND (MSA n=5, corticobasal degeneration [CBD] n=1, and DLB n=1). The genetic cohort consisted of *GBA* mutation carriers (unaffected

[n=127] and affected [n=64]); *LRRK2* mutation carriers (unaffected [n=166] and affected [n=135]) and *SNCA* mutation carriers (unaffected [n=5] and affected [n=17]). Genetic cohort subjects' visits until the 36-month follow-up were included (for the unaffected *GBA* mutation carriers the available follow-up was until the 24-month visit).

Data were retrieved from the PPMI data portal at the Laboratory of Neuroimaging (LONI) at the University of Southern California on 6.6.2019 (ppmi-info.org).

Sample processing and immunoassays for NfL quantification

CSF samples of the DeNoPa-cohort were quantified by the commercial enzyme linked immunoabsorbent assay (ELISA from Uman Diagnostics NfL ELISA kit, Umeå, Sweden). All other samples were analysed by the Simoa NF-L Kit for serum and Simoa Neuro 4-plex A kit for CSF (in the cross-sectional training cohort) with the fully automated SIMOA® HD-1 analyzer (Quanterix, Lexington, MA, USA). More details about processing of samples in the cohorts and assay validation have been published elsewhere[3, 16] and can be found in the online supplement or in the biologics manual for PPMI (http://ppmi-info.org).

Standard protocol approvals, registrations, and patient consent

Approval was received from the local ethical standards committee on human experimentation for all human participants. Written informed consent for research was obtained from all study participants. DeNoPa is registered in the German Register for Clinical trials (DRKS00000540), PPMI in clinicaltrials.gov as NCT01141023.

Statistical analysis

All analyses were performed with the statistical software R (version 3.6.0; R Core Team 2018), using the R-packages lme4 for the linear mixed effect Models (LME), and geepack for Generalized Estimation Equations (GEE) in the smaller DeNoPa-cohort to test for longitudinal changes over time. The significance level was set to alpha = 5% for all statistical tests. More details on the statistical analysis of the training cohorts can be found in the supplement.

Analysis of the PPMI validation cohort

Spearman rank based correlation coefficients between log10NFL at baseline and the different other predictors were calculated for the entire cohort and in the PD sub-cohort. Estimating the rate of change of log10NFL over time was done via LME modelling allowing for random intercepts only (some models with random slopes had convergence issues and hence we opted for the simpler ones). For all models we adjusted for age, diagnostic groups (PD, HC, OND etc.), sex, and levodopa equivalent daily doses (log10LEDD). Testing 13 variables simultaneously causes an inflation in the occurrence of type I errors that we accounted for by setting the significance level to 0·05/13~0·0038.

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The funders had no role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, in the preparation, review, or approval of the manuscript or in the decision to submit the manuscript for publication.

RESULTS

NfL measures in the training cohorts (DeNoPa- and Kassel cohorts)

Quantification of NfL was first carried out in CSF samples from the longitudinal DeNoPa-cohort. Demographics and baseline characteristics are shown in **supplementary table 1a**. The group difference showed higher values in PD [median 586 pg/ml and interquartile range (IRQ) as 331 pg/ml] vs. HC (median 507pg/ml and IRQ as 214 pg/ml) and OND (median 838 pg/ml and IRQ as 880 pg/ml). With a multiple regression model (**Supplementary table 1b**), we found a mean increase of 2·2% in CSF NfL levels with each yearly increase in age (p<0·0001). A woman's CSF NfL level is on average 0·84 times that of a man's (p=0·01828). Adjusting for age and sex mean CSF NfL levels were higher in patients with OND (91% higher) and PD (21% higher) than in HC (p<0·0001 and p=0·00963, respectively; **Figure 1a**).

Following a linear mixed model (**Supplementary table 1c**) adjusted for sex, baseline age, and baseline CSF NfL, a healthy subject has on average a 3·6% increase in CSF NfL levels each year (p>0·05). Compared with HC, the increment per year in CSF NfL levels is on average 1·03 times greater in PD (p>0·05), and 0·98 times greater in OND (p>0·05).

For the longitudinal association of CSF NfL with clinical covariates, we found a relationship between the longitudinal CSF NfL levels and the longitudinal progression of MDS-UPDRS III and total scores in the PD group (p=0·0231 and 0·0081).

In a second exploratory step, we analysed a cross-sectional cohort where CSF and serum NfL levels were measured; the cohort demographics are reported in-**Supplementary table 2a**. In the OND group, 17 subjects with MSA showed the highest levels of NfL in CSF and serum (data not shown). For the cross-sectional cohort (Kassel), using a multiple regression model

(**Supplementary table 2b**), on average there is a 3·1% increase in serum NfL (2·3% increase in CSF NfL) with each yearly increase in age (p<0·0001 for both). A woman's serum NfL level is on average 1·77 (0·91 for CSF) times that of a man's [p=0·0134 (serum) and p=0·1768 (CSF)]. Adjusting for age and sex, patients with OND have a mean serum NfL level 93% (123% for CSF) higher than that of HC (p<0·0001 both), while those with PD have a mean serum NfL level 94% (85% for CSF) higher than that of HC [p<0·0001 (serum) and p=0·0004 (CSF)].

The significant association of longitudinal NfL levels with clinical progression in PD and the strong correlation between CSF and serum NfL (by Spearman's rank $\hat{\rho} = 0 \cdot 73$; p < 10^-06; **Supplementary figure 1**) encouraged us to move to the validation step using serum samples from the longitudinal PPMI-cohort.

Serum NfL measures in the PPMI-cohort

The baseline characteristics of the PPMI-cohort are shown in **Table 1**. Among the 1131 subjects, the prodromal group has the highest median age (67·7 years), followed by the OND group, the genetic PD group, the HC, the PD group, and the unaffected mutation carriers (**Table 1**). The baseline serum NfL levels are highest in the prodromal and OND groups with a median of 14·5 pg/ml and an IQR as 7·2 pg/ml, and a median of 14·5 pg/ml and IQR as 10·3 pg/ml, respectively. The genetic PD group has a median serum NfL of 12·9 pg/ml (IQR=8·9 pg/ml), followed by the PD subjects (11·4 pg/ml, IQR=7·52 pg/ml), unaffected mutation carriers (11·2 pg/ml, IQR=6·09 pg/ml), and HC (10·4 pg/ml, IQR=7·51 pg/ml).

Spearman rank correlation between NfL and other variables at baseline in the PD group showed a high correlation with age (0·72); SDMT (-0·37), LNS (-0·26), MoCA (-0.19), Hopkins Verbal

Learning Tests (HVLT;-0·18), MDS-UPDRS total score (0·16), and BJLOS (-0·16) (all significant with p<0·05).

Cross-sectional serum NfL values at baseline in the PPMI-cohort

Multiple regression analysis shows that (**Table 2**) on average there is a 3% increase in serum NfL for each year of age (p=0·0003). A woman's serum NfL level is on average 1·05 times that of a man's (p<0·0001). Given the same age and sex, patients with OND have a mean serum NfL level 47% higher than that of HC (p=0·008), and those with genetic PD have a mean serum NfL level 21% higher than that of HC (p<0·0001). There are also non-statistically significant trends of increased serum NfL level in PD and prodromal groups compared with HC (6% higher with p=0.08 and 10% higher with p=0·09, respectively).

Longitudinal change of serum NfL over time in PPMI-cohort

Using a linear mixed model (**Table 3**) adjusted for sex, baseline age and baseline serum NfL, a healthy subject has an average increase of 3·2% in serum NfL each year (p<0·0001). Compared with HC, the increment rate is on average 1·06 times greater in PD (p<0.0001) and 1·46 times greater in OND (p<0·0001) (**Figure 1b**). The increment rate in the genetic cohort is not significantly different from that of HC (**Table 3; Figure 1c**).

Discussion

We investigated NfL levels by sandwich immunoassay in longitudinal and cross-sectional training cohorts from a single centre and validated the findings in a longitudinal multicentre cohort consisting of HC, prodromal and established PD. We also studied symptomatic and

asymptomatic mutation carriers of known PD genetic mutations with follow-ups of up to six years. Results obtained from our training cohorts indicated that serum and CSF NfL levels were higher in PD subjects compared with controls; levels were higher in OND compared with PD. CSF NfL levels increased longitudinally over four years and correlated with MDS-UPDRS III and total scores.

In the second step, we focused on NfL serum levels in the validation cohort, considering the strong correlation between CSF and serum NfL levels and the advantage of using a peripheral and less invasive source for the specimen. Consistent with the exploratory work in the training cohorts, the main findings in the validation cohort were: (1) mean levels of serum NfL were higher in established PD versus HC at each of the six timepoints during the first five years after diagnosis; (2) markedly higher levels of NfL were seen in the few subjects with other neurodegenerative disorders, including subjects with DLB and MSA (discussed in more detail below) (3) there was a significant (age-adjusted) longitudinal increase in serum NfL in the PD compared to HC and (4) the longitudinal change in serum NfL correlated significantly with MDS-UPDRS III motor and total scores, suggesting that serum NfL may be a biomarker of clinical progression. Overall, the longitudinal analysis of serum NfL in the validation set confirmed the findings from the training cohorts. The PPMI cohort enabled us to analyse prodromal conditions and a genetic cohort with symptomatic and asymptomatic mutation carriers. We found that the serum NfL levels of subjects with two prodromal conditions for α-synuclein aggregation disorders feature higher than PD or HC and lower than OND, including MSA and DLB. This was as expected because iRBD subjects especially have a high risk to predominantly convert into PD but also to DLB and MSA. In the genetic cohort, serum NfL levels of asymptomatic mutation carriers in all three mutation carrier groups were lower than in

their respective symptomatic group. The levels in all six genetic groups (symptomatic and asymptomatic) remained relatively stable over the two to three years of follow-up and there were no differences in NfL rate of change between the mutation carrier groups. We also observed a higher level of serum NfL in symptomatic mutation carriers relative to PD patients, possibly reflecting a longer duration of disease. Subsequent analyses will explore the effects of disease duration on changes in serum NfL.

Elevated levels of NfL, as seen here in PD and OND compared with HC, have been identified in several other neurological conditions including dementia disorders and multiple sclerosis. Therefore, this marker for axonal damage is not specific for any disease,[7] but could be useful for exploring specific questions within disease entities. Within PD spectrum disorders, NfL may be particularly of use in discriminating PD from cognate disorders such as MSA, PSP, and DLB, as has been previously described.[12] Due to the high rate of misdiagnoses in early PD as shown in a neuropathologically confirmed cohort,[2] there is a need for biomarkers to distinguish sporadic PD from other neurodegenerative disorders with Parkinson syndromes, such as the two α-synuclein aggregation disorders DLB with early dementia and marked hallucinations and MSA with clinically severe autonomic disturbances and α-synuclein deposition in the glia (and not in neurons), thus, disorders with a completely different clinical and neuropathological disease phenotype. This is important in clinical practice as “Parkinson-plus” syndromes may have a completely different underlying pathophysiology, have worse prognoses and may ultimately require different therapy regimens. This need will grow as medicine becomes more personalized. In addition, the ability to select patients for inclusion or stratification within clinical trials is also becoming increasingly important. The diagnosis of PD in PPMI is clinical; it is based on systematic history and examination, structural magnetic resonance imaging (to rule out other

diseases), pathological DaT by central read, and most importantly by longitudinal clinical follow-up. Based on this process, seven subjects in the PD cohort analysed here were found to have diagnoses other than PD after five years follow-up and were thus taken out of the PD cohort. This group of subjects, despite the small size, was separated in the analysis as OND. Serum NfL levels in this OND group were higher at baseline (before the follow-up and before evolving into another disease) and may provide future diagnostic utility. Additional studies including analysis on samples from post-mortem PD confirmed subjects will further establish the utility of serum NfL measurements in differentiating PD from OND. In numerous previous CSF studies (conducted before ultrasensitive technologies were available) the mean NfL levels in other neurological disorders were found to be markedly increased compared to HC, such as in multiple sclerosis (4·5-fold increase), traumatic brain injury (three-fold increase), PSP, CBD, MSA (3-4·25 fold increase) as well as in other, more slowly progressing neurodegenerative disorders such as Alzheimer's disease (1·5-2-fold increase).[17] Compared to these disorders, the increase is smaller in PD (1·6 in CSF and 1·25 in serum), where α-synuclein aggregation occurs mainly in neurons with high energy turnover in less myelinated axons,[18] while NfL is mainly expressed in larger myelinated axons.[19] Also, in contrast to many more rapidly progressive or acute diseases, the pathological changes in PD and the respective cell loss is only mild.[20] Despite the slight increase shown here, the relatively higher serum NfL levels in the prodromal groups (compared to PD and HC), especially in the iRBD group, may indicate potential for conversion to either PD or Parkinsonian syndromes. Furthermore, longitudinal NfL levels may, in fact, be highest in the early stages of the disease with the greatest disease activity as has been similarly seen in β-amyloid in Alzheimer's disease.[21]

Another variable affecting the increase of NfL levels is age. Across published studies, NfL in CSF and blood increases with age[17] as we also identified in our cohorts (also shown in **Supplementary Figure 2**). For example, the prodromal groups in PPMI are on average 3-5 years older than the PD group and the iRBD subjects are older than the hyposmic subjects featuring higher NfL values. Age is likely also the main reason why asymptomatic *SNCA* mutation carriers have the lowest levels in the PPMI cohort—the mean age in this group is 42±4·6 years. The reasons for this positive association of serum NfL and age is explained by structural alterations of the axons with ageing, including vascular disease, metabolic changes, and inflammation. All of these have also been shown to play a role in PD progression[22], which may also influence the NfL levels in blood.

In contrast to a previous report showing that CSF NfL levels were relatively stable despite disease progression in PD over 12 months[14] we have observed an increase of serum NfL over 72 and 60 months in the training and validation cohorts, respectively. The longitudinal increase in serum NfL significantly correlated with changes over time in MDS-UPDRS III and total scores. A significant association with longitudinal progression of serum NfL was also seen for the processing speed/attention test SDMT and the global cognitive Screen MoCa (**supplementary table 3**).

We did not observe a longitudinal change of serum NfL in the prodromal cohort. This could be due to the heterogeneity of the group as recruited, both in terms of disease stage as well as eventual diagnostic category and phenotypes of individual subjects. The apparent stability over time in the prodromal cohort could also indicate that serum NfL levels do not change continuously before motor symptoms evolve. This suggestion is also supported by the longitudinal stability of serum NfL in the asymptomatic mutation carriers. Only continued

follow-up will identify subjects converting to a motor disease or symptomatic disease state. The individual levels may indicate the prognostic direction.

In conclusion, we have identified NfL as the first blood-based PD progression biomarker. We observed slightly higher levels of serum NfL in PD compared to HC even at early stages of the disease, a mild longitudinal increase in serum NfL levels over time and correlations of serum NfL with clinical measures of disease progression in two independent longitudinal cohorts. Increases in serum NfL were, in general, less than those seen in OND, which are either more rapidly progressive than PD, associated with damage to myelinated tracts (as seen in multiple sclerosis), or associated with significantly greater cell death (as seen in Alzheimer's and extreme in Creutzfeldt-Jakob disease). Our NfL data will be strengthened with continued analyses of these data using additional alternative statistical models as well as follow-up of the cohorts, especially in subjects at risk for disease progression. In addition, monitoring of larger longitudinal cohorts with a focus on prodromal or asymptomatic PD with longer observational time to allow the development of motor disease are needed. This being said, we remain cognizant that increased NfL levels are not specific to PD or any other neurodegenerative disorder. Thus, more specific markers will need to be identified, leading hopefully to a panel of different markers reflecting disease state, rate, and fate. Finally, we note the profound influence of age and gender on serum NfL levels. We, therefore, recommend that age-and sex-based adjustments be applied when interpreting serum NfL levels in clinical research and practice.

Research in context

Systematic Review

We searched PubMed and Medline databases for English language original articles with the terms “Parkinson’s disease”, “Parkinson disease” and “serum” or “cerebrospinal fluid”, and “neurofilament light chain” or “NFL”.

Interpretation

To date several teams have carried out cross-sectional studies to quantify neurofilament light chain (NfL) in cerebrospinal fluid (CSF) and in blood in Parkinson’s disease (PD). Some reports showed normal or slightly elevated levels in PD. In contrast, several studies showed elevated levels of CSF and/or serum NfL in other Parkinson syndromes that feature Parkinsonism as a symptom but have an underlying other disorder, mostly more progressive neurodegenerative processes that PD, such as Multiple System Atrophy (MSA), Progressive Supranuclear Palsy (PSP) or Dementia with Lewy Bodies (DLB). These studies have been reviewed recently.[17]

One longitudinal study on CSF NfL[14] showed stable levels over follow-up but based on the cross sectional studies mentioned above a positive correlation was demonstrated between CSF NfL and Hoehn and Yahr PD severity stages and the motor part of the commonly used Unified PD Rating Scale (UPDRS-III). One study demonstrated a positive correlation with the levodopa equivalent daily dosage. A first study on plasma NfL, that was recently published showed in a small longitudinal cohort with two timepoints (baseline and 3.4±1.2 years follow-up) a modest correlation of plasma NfL with the MDS-UPDRS III.[23]

We analyzed samples from three different and independent cohorts including the Parkinson Progression Marker Initiative with 1131 longitudinal serum samples, that comprise of manifest, early PD participants and healthy controls (HC) as well as subjects with prodromal conditions (isolated hyposmia or REM sleep behavior disorder) who present a high risk of conversion into mostly α-synuclein related neurodegenerative disorders, and a genetic cohort of symptomatic and asymptomatic mutation carriers at risk of developing PD. We found that serum NfL is higher in established PD versus HC; There was a significant (age-adjusted) longitudinal increase in serum NfL in PD compared to HC and the longitudinal change in serum NfL correlated significantly with MDS-UPDRS III motor and total scores, suggesting that serum NfL may be a biomarker of clinical progression.

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Author contributions:

BM, DG, and JMC conceived the study. BM, SH, and DG designed the study and were responsible for data processing; MD, TYL, FG, WW, RG, DG and BM oversaw all statistical analyses; NK, HZ and DG were involved in sample analyses and data interpretation; MF, KM, LMC, SH and AS oversaw patient recruitment and assisted in the interpretation of data. BM wrote the manuscript. MD, TYL, WW, FG, DG, TF, HZ, SS, RG, NK, MF, LMC, TS, ABS, DW, KM, AS, JMC, SH, DG, CMT and CT co-edited the manuscript. BM, SH, DG, and MD had full access to the clinical primary data and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors had access to the data generated in the study including the statistical analysis and decided to submit the paper for publication.

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FIGURE LEGENDS

Figure 1a: Mean of log10 transformed serum NfL levels at each visit in healthy controls (HC), Parkinson's disease (PD) and other neurodegenerative disorders (OND) in the DeNoPa-cohort (demographics in Supplementary Table 1).
The dashed line represents a linear fit through the points and the vertical bars give estimates of the errors.

Figure 1b: Mean of log10 transformed serum NfL levels at each visit in the core and prodromal group: healthy controls (HC), Parkinson's disease (PD), other neurodegenerative disorders (OND), hyposmics and subjects with isolated REM sleep behaviour disorder (iRBD) in the PPMI-cohort (demographics in Table 1).
The dashed line represents a linear fit through the points and the vertical bars give estimates of the errors.

Figure 1c: Mean of log10 transformed serum NfL levels at each visit in the genetic cohort (GC): unaffected (UN) and affected (PD) LRRK2, GBA and SNCA mutation carriers (demographics in Table 1). The dashed line represents a linear fit through the points and the vertical bars give estimates of the errors.

Table 1: Demographics, clinical, dopamine transporter imaging and baseline serum NfL data in the core group and the genetic cohort of the validation cohort

		Core Group					Genetic Cohort					
							affected			unaffected		
Parameter	**Level**	**Healthy Controls (HC)**	**Parkinson's Disease (PD)**	**Other neuro degenerative disorder (OND)**	**Hyposmia**	**isolated RBD (iRBD)**	**PD-GBA**	**PD-LRRK2**	**PD-SNCA**	**UN-GBA**	**UN-LRRK2**	**UN-SNCA**
n		178	375	7	22	35	64	135	17	127	166	5
Sex												
	female	63 (35·4%)	137 (36·5%)	2 (28·6%)	6 (27·3%)	6 (17·1%)	29 (45·3%)	75 (55·1%)	8 (47·1%)	80 (62·0%)	95 (57·2%)	4 (80·0%)
	male	115 (64·6%)	238 (63·5%)	5 (71·4%)	16 (72·7%)	29 (82·9%)	35 (54·7%)	61 (44·9%)	9 (52·9%)	49 (38·0%)	71 (42·8%)	1 (20·0%)
Age (years)												
	mean ± sd	61 ± 11	62 ± 9·8	64 ± 7	68 ± 6·4	70 ± 5·5	63 ± 9·6	64 ± 9·4	48 ± 9·4	62 ± 6·9	62 ± 7·1	42 ± 4·6
	median (min; max)	62 (31; 84)	62 (34; 85)	66 (53; 73)	67 (61; 83)	70 (59; 82)	66 (32; 81)	65 (34; 85)	50 (32; 69)	62 (45; 84)	62 (49; 82)	44 (34; 45)
Duration (years)												
	mean ± sd	NA	0·56 ± 0·56	0·63 ± 0·69	NA	NA	3·2 ± 2·1	3·1 ± 2·1	3·4 ± 2·2	NA	NA	NA
	median (min; max)	NA	0·33 (0; 3)	0·5 (0·079; 2)	NA	NA	3 (0·082;7·2)	2·6 (0·082;8·7)	3·6 (0·17; 6·1)	NA	NA	NA
LEDD												
	mean ± sd	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	median (min; max)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)
Serum NFL (pg/ml)												

	mean ± sd	12 ± 6·7	13 ± 7·2	18 ± 7	15 ± 5·1	17 ± 8	16 ± 11	16 ± 9·9	13 ± 9·9	13 ± 7·2	13 ± 6·2	5·7 ± 3·4
	median (min; max)	10 (2·4; 51)	11 (1·8; 77)	14 (12; 29)	15 (6·5; 26)	14 (6·8; 39)	13 (3·8; 81)	13 (3·9; 71)	9·7 (5·7; 41)	12 (3·9; 63)	11 (2·3; 47)	5·3 (2; 11)
MDS-UPDRS III												
	mean ± sd	1·2 ± 2·3	21 ± 8·9	27 ± 5·8	2·7 ± 3·1	4·5 ± 4	27 ± 11	21 ± 11	14 ± 7·3	2·4 ± 3·7	3 ± 4·1	0 ± 0
	median (min; max)	0 (0; 13)	20 (4; 51)	27 (18; 36)	1 (0; 10)	3 (0; 15)	26 (7; 48)	19 (4; 56)	12 (6; 23)	1 (0; 21)	2 (0; 27)	0 (0; 0)
MDS-UPDRS Total Score												
	mean ± sd	4·7 ± 4·5	32 ± 13	46 ± 11	10 ± 7·2	14 ± 7·2	44 ± 16	37 ± 18	27 ± 11	9·1 ± 7·9	8·5 ± 7·7	3·4 ± 2·7
	median (min; max)	3 (0; 20)	31 (7; 70)	44 (37; 68)	8 (0; 26)	14 (3; 31)	41 (17; 77)	35 (7; 113)	28 (9; 41)	7 (0; 44)	6·5 (0; 45)	3 (1; 8)
TD/PIGD												
	TD	9 (5·1%)	65 (17·3%)	4 (57·1%)	4 (18·2%)	10 (28·6%)	18 (35·3%)	46 (46·5%)	3 (37·5%)	19 (15·0%)	18 (10·8%)	0 (0·0%)
	intermediate	144 (80·9%)	41 (10·9%)	3 (42·9%)	12 (54·5%)	14 (40·0%)	3 (5·9%)	7 (7·1%)	1 (12·5%)	73 (57·5%)	109 (65·7%)	5 (100·0%)
	PIGD	25 (14·0%)	269 (71·7%)	0 (0·0%)	6 (27·3%)	11 (31·4%)	30 (58·8%)	46 (46·5%)	4 (50·0%)	35 (27·6%)	39 (23·5%)	0 (0·0%)
DaT-Scan caudate (SBR)												
	mean ± sd	3 ± 0·6	2 ± 0·54	1·5 ± 0·67	NA	NA	1·9 ± 0·74	1·8 ± 0·5	1·3 ± 0·56	NA	NA	NA
	median (min; max)	2·9 (1·3; 4·8)	2 (0·39; 3·7)	1·4 (0·74; 2·6)	NA	NA	1·8 (0·43; 3·9)	1·9 (0·68; 3)	1·1 (0·78; 2·5)	NA	NA	NA

DaT-Scan putamen (SBR)												
	mean ± sd	2·1 ± 0·54	0·82 ± 0·28	0·65 ± 0·35	NA	NA	0·8 ± 0·45	0·76 ± 0·3	0·6 ± 0·27	NA	NA	NA
	median (min; max)	2·1 (0·64;3·9)	0·79 (0·24; 2·2)	0·56 (0·31; 1·3)	NA	NA	0·7 (0·27; 2·4)	0·73 (0·21; 2·1)	0·46 (0·27; 1)	NA	NA	NA
DaT-Scan striatum (SBR)												
	mean ± sd	2·6 ± 0·55	1·4 ± 0·39	1·1 ± 0·49	NA	NA	1·3 ± 0·57	1·3 ± 0·38	0·97 ± 0·4	NA	NA	NA
	median (min; max)	2·5 (0·98;4·2)	1·4 (0·31; 2·6)	0·99 (0·53; 1·9)	NA	NA	1·2 (0·38; 3·1)	1·3 (0·45; 2·5)	0·77 (0·52; 1·8)	NA	NA	NA
MoCA												
	mean ± sd	28 ± 1·1	27 ± 2·4	27 ± 2·3	28 ± 1·6	26 ± 4·3	26 ± 2·8	26 ± 3·2	25 ± 5·7	27 ± 2·3	27 ± 2·3	26 ± 2·5
	median (min; max)	28 (27; 30)	27 (17; 30)	28 (23; 30)	28 (24; 30)	27 (11; 30)	27 (15; 30)	27 (13; 30)	27 (11; 30)	27 (16; 30)	27 (20; 30)	28 (23; 28)
SDMT												
	mean ± sd	47 ± 11	41 ± 9·7	38 ± 8·2	42 ± 11	32 ± 9·4	38 ± 12	39 ± 12	32 ± 17	47 ± 7·7	45 ± 10	50 ± 7·7
	median (min; max)	46 (20; 83)	42 (7; 82)	41 (23; 46)	45 (16; 55)	32 (15; 56)	40 (9; 65)	40 (10; 75)	31 (6; 73)	47 (24; 68)	46 (14; 74)	51 (39; 59)
LNS												
	mean ± sd	11 ± 2·6	11 ± 2·7	10 ± 1·5	10 ± 1·8	9 ± 3·5	10 ± 2·9	9·7 ± 3	7·1 ± 2·8	12 ± 2·9	11 ± 2·7	12 ± 0·84
	median (min; max)	11 (2; 20)	11 (2; 20)	10 (9; 13)	10 (6; 14)	9 (3; 17)	10 (4; 17)	10 (2; 18)	8 (2; 12)	11 (0; 19)	11 (4; 20)	12 (11; 13)
HVLT-IR												
	mean ± sd	26 ± 4·6	25 ± 4·9	26 ± 4·9	23 ± 5·5	21 ± 5·1	24 ± 5·1	24 ± 5·3	20 ± 7·6	27 ± 4·4	26 ± 5·3	28 ± 5·4
	median (min; max)	27 (15; 35)	25 (9; 36)	25 (18; 32)	23 (12; 33)	21 (9; 33)	25 (12; 33)	25 (8; 34)	22 (9; 31)	28 (14; 36)	27 (9; 35)	28 (20; 34)
HVLT-DG												

	mean ± sd	10 ± 2·9	9·7 ± 2·5	9·9 ± 1·9	10 ± 1·5	8·9 ± 2·4	10 ± 1·7	10 ± 2·4	8·9 ± 3·3	11 ± 1·4	11 ± 2·1	11 ± 1·1
	median (min; max)	11 (-4; 12)	10 (-2; 12)	10 (7; 12)	11 (5; 12)	10 (2; 12)	10 (3; 12)	11 (-1; 12)	10 (0; 12)	11 (5; 12)	11 (0; 12)	12 (10; 12)
HVLT-RT												
	mean ± sd	0·91±0·18	0·85 ± 0·2	0·87 ± 0·18	0·8 ± 0·3	0·77±0·28	0·83± 0·26	0·82± 0·24	0·72± 0·35	0·88± 0·17	0·89± 0·16	0·92± 0·18
	median (min; max)	0·92 (0·27; 1·5)	0·9 (0; 1·3)	0·8 (0·71; 1·2)	0·91 (0; 1·1)	0·82 (0; 1·2)	0·9 (0; 1·4)	0·89 (0; 1·2)	0·8 (0; 1·3)	0·92 (0·29; 1·4)	0·92 (0·12; 1·2)	0·92 (0·73; 1·1)
BJLOS												
	mean ± sd	13 ± 2	13 ± 2·1	13 ± 2·3	13 ± 2	11 ± 2·4	12 ± 2·9	12 ± 3	9·9 ± 3·2	13 ± 1·9	13 ± 2	12 ± 2·8
	median (min; max)	14 (4; 15)	13 (5; 15)	13 (8; 15)	13 (8; 15)	11 (3; 15)	12 (3; 15)	12 (0; 15)	10 (6; 15)	14 (8; 15)	13 (5; 15)	13 (8; 15)

Abbreviations: LEDD: Levodopa equivalent daily dosage; education adjusted Montreal Cognitive Assessment (MoCA); Symbol Digit Modality Test (SDMT), executive function/working memory (WMS-III); Letter-Number Sequencing Test (LNS); Benton Judgment of Line Orientation test (BJLO); Movement Disorders Society Unified Parkinson's Disease rating Scale (MDS-UPDRS); Tremor dominant/Postural instability and gait subtype (TD/PIGD); Dopamine transporter imaging (DaT-Scan); Striatal Binding Ratio (SBR); Neurofilament light chain (NfL); Hopkins Verbal Learning Tests with immediate/total recall (HVLT-IR), discrimination recognition (HVLT-DG) and retention (HVLT-RT)

Table 2: Multiple regression analysis of log10 baseline NfL levels in serum in the PPMI-cohort (demographics in table 1)

	Estimate	**Standard Error**	***p*-value**
Intercept	0·5615	0·0205	**<0·0001**
Age (years)	0·0146	0·0005	**<0·0001**
Gender (female)	0·0209	0·0100	**0·0371**
Genetic Cohort-Unaffected	0·0224	0·0156	0·1501
Prodromal	0·0430	0·0251	0·0875
Parkinson's Disease	0·0262	0·0148	0·0775
Genetic Cohort-PD	0·0826	0·0165	**<0·0001**
Other Neurodegenerative Diseases	0·1670	0·0627	**0·0078**

*Age was centered at 30 years old.
*Healthy controls and male are the references of the categorical variables.
*Multiple regression R^2=0·435

Table 3: Linear mixed model of log10 changes of serum NfL over time based on the PPMI cohort.
The mixed model contained random intercepts only.

parameter	level	BL	V02	V04	V06	V08	V12	p-value	test	adjusted p-value
Healthy Controls (HC; n)		**178**	**163**	**140**	**149**	**146**				
MDS-UPDRS III								0.01	Kruskal-Wallis rank sum test	
	mean ± sd	1.2 ± 2.3	1.6 ± 2.8	1.2 ± 2.2	1.6 ± 3.6	2.3 ± 3.9				0.03
	median (min; max)	0 (0; 13)	0 (0; 20)	0 (0; 11)	0 (0; 26)	1 (0; 31)				
MDS-UPDRS total score								0.04	Kruskal-Wallis rank sum test	
	mean ± sd	4.7 ± 4.5	5.3 ± 5.2	4.8 ± 4.4	5.5 ± 5.4	6.6 ± 6.3				0.16
	median (min; max)	3 (0; 20)	4 (0; 25)	4 (0; 23)	4 (0; 27)	5 (0; 38)				
NFL								0.07	Kruskal-Wallis rank sum test	
	mean ± sd	12 ± 6.7	12 ± 7.5	12 ± 5.7	13 ± 6.5	13 ± 6.5				0.21
	median (min; max)	10 (2.4; 51)	11 (2.3; 71)	11 (3.3; 30)	12 (3.5; 37)	12 (3.5; 48)				
Parkinson's Disease (PD; n)		**375**	**239**	**182**	**262**	**290**	**273**			
MDS-UPDRS III								< 0.01	Kruskal-Wallis rank sum test	
	mean ± sd	21 ± 8.9	24 ± 9.4	21 ± 10	22 ± 11	23 ± 12	24 ± 13			< 0.01
	median (min; max)	20 (4; 51)	23 (3; 56)	20 (1; 50)	21 (0; 56)	22 (0; 65)	24 (0; 85)			
MDS-UPDRS total score								< 0.01	Kruskal-Wallis rank sum test	
	mean ± sd	32 ± 13	36 ± 14	35 ± 15	37 ± 16	40 ± 17	43 ± 20			< 0.01
	median (min; max)	31 (7; 70)	36 (4; 94)	33 (4; 83)	35 (5; 81)	38 (3; 106)	41 (5; 156)			
NFL								< 0.01	Kruskal-Wallis rank sum test	

	mean ± sd	13 ± 7.2	14 ± 10	15 ± 12	15 ± 9.5	16 ± 9.9	19 ± 15			< 0.01
	median (min; max)	11 (1.8; 77)	12 (4; 99)	13 (2.2; 131)	13 (3.4; 108)	14 (2.2; 102)	15 (3.4; 131)			
Other neurological disorders (OND; n)		**7**	**4**	**6**	**5**	**6**	**5**			
MDS-UPDRS III								0.02	Kruskal-Wallis rank sum test	
	mean ± sd	27 ± 5.8	32 ± 17	30 ± 5.6	36 ± 6.5	42 ± 9.1	52 ± 16			0.16
	median (min; max)	27 (18; 36)	32 (13; 52)	30 (22; 37)	35 (28; 46)	44 (29; 53)	56 (30; 68)			
MDS-UPDRS total score								< 0.01	Kruskal-Wallis rank sum test	
	mean ± sd	46 ± 11	57 ± 25	58 ± 8.8	67 ± 7.2	82 ± 20	102 ± 29			0.01
	median (min; max)	44 (37; 68)	52 (34; 91)	60 (45; 68)	63 (59; 75)	76 (62; 118)	96 (69; 143)			
NFL								0.03	Kruskal-Wallis rank sum test	
	mean ± sd	18 ± 7	17 ± 5.6	24 ± 6.6	36 ± 14	27 ± 9.1	59 ± 64			0.20
	median (min; max)	14 (12; 29)	16 (12; 25)	25 (16; 32)	33 (24; 58)	26 (17; 39)	30 (19; 171)			
Hyposmia (n)		**22**	**24**	**23**	**21**	**20**				
MDS-UPDRS III								0.27	Kruskal-Wallis rank sum test	
	mean ± sd	2.7 ± 3.1	4.2 ± 7.1	6.1 ± 8.7	6.5 ± 5.8	7.4 ± 8.6				1.0
	median (min; max)	1 (0; 10)	2 (0; 33)	2 (0; 36)	7 (0; 17)	1.5 (0; 27)				
MDS-UPDRS total score								0.48	Kruskal-Wallis rank sum test	
	mean ± sd	10 ± 7.2	11 ± 9.8	13 ± 13	14 ± 10	15 ± 12				1.0
	median (min; max)	8 (0; 26)	7 (1; 38)	8 (0; 45)	13 (1; 37)	11 (1; 36)				
NFL								0.95	Kruskal-Wallis rank sum test	

	mean ± sd	15 ± 5.1	16 ± 5.6	16 ± 6.3	25 ± 43	18 ± 9				1.0
	median (min; max)	15 (6.5; 26)	15 (6.3; 32)	14 (7.9; 34)	14 (7.5; 210)	16 (8.7; 46)				
isolated REM Sleep Behaviour Disorder (iRBD; n)		**35**	**34**	**36**	**36**	**32**				
MDS-UPDRS III								0.11	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	4.5 ± 4 3 (0; 15)	4.8 ± 4.6 3 (0; 18)	7.6 ± 9.3 4.5 (0; 41)	8 ± 9.5 6 (0; 49)	11 ± 13 7.5 (0; 51)				0.45
MDS-UPDRS total score								0.06	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	14 ± 7.2 14 (3; 31)	13 ± 7.6 10 (2; 29)	18 ± 15 15 (3; 63)	20 ± 15 14 (2; 74)	24 ± 21 16 (3; 86)				0.28
NFL								0.25	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	17 ± 8 14 (6.8; 39)	19 ± 14 16 (6.5; 91)	17 ± 8.3 15 (6.4; 50)	19 ± 10 16 (4.9; 49)	18 ± 6.2 17 (6; 34)				0.76
PD-GBA (n)		**64**	**63**	**48**	**24**	**2**				
MDS-UPDRS III								0.63	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	23 ± 12 23 (4; 54)	24 ± 12 22 (7; 64)	22 ± 11 20 (4; 51)	21 ± 10 19 (0; 44)	12 ± 11 12 (4; 20)				1.0
MDS-UPDRS total score								0.96	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	40 ± 16 36 (14; 77)	42 ± 18 39 (15; 98)	41 ± 17 38 (13; 85)	40 ± 13 42 (6; 63)	35 ± 20 35 (21; 49)				1.0
NFL								0.65	Kruskal-Wallis rank sum test	

	mean ± sd	16 ± 11	18 ± 13	17 ± 11	21 ± 24	17 ± 3.5				1.0
	median (min; max)	13 (3.8; 81)	14 (5; 96)	14 (4.3; 72)	14 (7.8; 130)	17 (14; 19)				
PD-LRRK2 (n)		**135**	**126**	**110**	**79**	**70**				
MDS-UPDRS III								0.35	Kruskal-Wallis rank sum test	
	mean ± sd	18 ± 9.4	20 ± 10	18 ± 10	19 ± 9.6	17 ± 8.8				1.0
	median (min; max)	16 (3; 48)	18 (2; 56)	17 (2; 59)	18 (2; 45)	17 (0; 44)				
MDS-UPDRS total score								0.36	Kruskal-Wallis rank sum test	
	mean ± sd	34 ± 16	37 ± 17	33 ± 16	35 ± 16	33 ± 14				1.0
	median (min; max)	32 (6; 80)	34 (8; 113)	30 (7; 100)	35 (6; 94)	32 (6; 70)				
NFL								0.66	Kruskal-Wallis rank sum test	
	mean ± sd	16 ± 9.9	17 ± 13	18 ± 17	48 ± 275	22 ± 57				1.0
	median (min; max)	13 (3.9; 71)	13 (4.4; 90)	14 (5.2; 163)	15 (5; 2463)	14 (6; 486)				
PD-SNCA (n)		**17**	**15**	**17**	**8**	**7**				
MDS-UPDRS III								0.28	Kruskal-Wallis rank sum test	
	mean ± sd	23 ± 20	25 ± 14	24 ± 17	23 ± 14	39 ± 23				0.34
	median (min; max)	14 (3; 71)	23 (7; 52)	17 (4; 65)	20 (6; 51)	29 (16; 80)				
MDS-UPDRS total score								0.10	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	42 ± 30 33 (8; 102)	49 ± 26 50 (12; 107)	52 ± 31 38 (23; 127)	52 ± 32 40 (22; 121)	78 ± 39 55 (44; 135)				0.31
NFL								0.17	Kruskal-Wallis rank sum test	
	mean ± sd	13 ± 9.9	20 ± 24	19 ± 13	20 ± 14	21 ± 19				0.34
	median (min;	9.7 (5.7;	12 (6.6;	15 (6.3; 54)	14 (9.8; 51)	15 (7.7; 64)				

	max)	41)	103)							
UN-GBA (n)		**127**	**113**	**95**	**42**					
MDS-UPDRS III								0.01	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	2.4 ± 3.7 1 (0; 21)	3.1 ± 4.4 2 (0; 28)	2.9 ± 3.5 2 (0; 17)	4.1 ± 4.3 3 (0; 22)					0.02
MDS-UPDRS total score								0.21	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	9.1 ± 7.9 7 (0; 44)	10 ± 8.8 8 (0; 50)	9.8 ± 8.4 8 (0; 47)	12 ± 8.6 9 (0; 36)					0.21
NFL								0.07	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	13 ± 7.2 12 (3.9; 63)	14 ± 7 12 (4.3; 47)	16 ± 17 12 (5.6; 168)	15 ± 6.7 15 (4.3; 36)					0.14
UN-LRRK2 (n)		**166**	**141**	**137**	**98**	**52**				
MDS-UPDRS III								0.49	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	3 ± 4.1 2 (0; 27)	3.3 ± 4.3 2 (0; 21)	3.6 ± 4.6 2 (0; 27)	3.3 ± 4.7 2 (0; 25)	3.8 ± 4.2 4 (0; 19)				1.0
MDS-UPDRS total score								0.67	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	8.5 ± 7.7 6.5 (0; 45)	8.7 ± 7.3 7 (0; 42)	9.7 ± 8.8 8 (0; 47)	9.4 ± 11 6 (0; 64)	9.1 ± 7 8 (0; 27)				1.0
NFL								0.84	Kruskal-Wallis rank sum test	
	mean ± sd	13 ± 6.2	14 ± 8.6	13 ± 6.7	13 ± 5.6	14 ± 7.1				1.0
	median (min; max)	11 (2.3; 47)	12 (3.6; 88)	12 (3.2; 50)	12 (3.3; 30)	11 (3.2; 41)				
UN-SNCA (n)		**5**	**5**	**5**	**3**	**3**				

MDS-UPDRS III								NaN	Kruskal-Wallis rank sum test	
	mean ± sd	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0				NaN.0
	median (min; max)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)				
MDS-UPDRS total score								0.90	Kruskal-Wallis rank sum test	
	mean ± sd	3.4 ± 2.7	2.6 ± 2.1	4 ± 2	3 ± 2	4.3 ± 4.9				1.0
	median (min; max)	3 (1; 8)	3 (0; 5)	4 (2; 6)	3 (1; 5)	2 (1; 10)				
NFL								0.93	Kruskal-Wallis rank sum test	
	mean ± sd	5.7 ± 3.4	6.2 ± 3.5	5.6 ± 2.1	8.2 ± 4.8	6.2 ± 3.2				1.0
	median (min; max)	5.3 (2; 11)	5.2 (3.2; 12)	5.5 (3.3; 8.6)	6.6 (4.3; 14)	5.8 (3.2; 9.5)				

parameter	level	BL	V02	V04	V06	V08	V12	p-value	test	adjusted p-value
Healthy Controls (HC; n)		**178**	**163**	**140**	**149**	**146**				
MDS-UPDRS III								0.01	Kruskal-Wallis rank sum test	
	mean ± sd	1.2 ± 2.3	1.6 ± 2.8	1.2 ± 2.2	1.6 ± 3.6	2.3 ± 3.9				0.03
	median (min; max)	0 (0; 13)	0 (0; 20)	0 (0; 11)	0 (0; 26)	1 (0; 31)				
MDS-UPDRS total score								0.04	Kruskal-Wallis rank sum test	
	mean ± sd	4.7 ± 4.5	5.3 ± 5.2	4.8 ± 4.4	5.5 ± 5.4	6.6 ± 6.3				0.16
	median (min; max)	3 (0; 20)	4 (0; 25)	4 (0; 23)	4 (0; 27)	5 (0; 38)				
NFL								0.07	Kruskal-Wallis rank sum test	
	mean ± sd	12 ± 6.7	12 ± 7.5	12 ± 5.7	13 ± 6.5	13 ± 6.5				0.21
	median (min; max)	10 (2.4; 51)	11 (2.3; 71)	11 (3.3; 30)	12 (3.5; 37)	12 (3.5; 48)				

Parkinson's Disease (PD; n)		**375**	**239**	**182**	**262**	**290**	**273**			
MDS-UPDRS III								< 0.01	Kruskal-Wallis rank sum test	
	mean ± sd	21 ± 8.9	24 ± 9.4	21 ± 10	22 ± 11	23 ± 12	24 ± 13			< 0.01
	median (min; max)	20 (4; 51)	23 (3; 56)	20 (1; 50)	21 (0; 56)	22 (0; 65)	24 (0; 85)			
MDS-UPDRS total score								< 0.01	Kruskal-Wallis rank sum test	
	mean ± sd	32 ± 13	36 ± 14	35 ± 15	37 ± 16	40 ± 17	43 ± 20			< 0.01
	median (min; max)	31 (7; 70)	36 (4; 94)	33 (4; 83)	35 (5; 81)	38 (3; 106)	41 (5; 156)			
NFL								< 0.01	Kruskal-Wallis rank sum test	
	mean ± sd	13 ± 7.2	14 ± 10	15 ± 12	15 ± 9.5	16 ± 9.9	19 ± 15			< 0.01
	median (min; max)	11 (1.8; 77)	12 (4; 99)	13 (2.2; 131)	13 (3.4; 108)	14 (2.2; 102)	15 (3.4; 131)			
Other neurological disorders (OND; n)		**7**	**4**	**6**	**5**	**6**	**5**			
MDS-UPDRS III								0.02	Kruskal-Wallis rank sum test	
	mean ± sd	27 ± 5.8	32 ± 17	30 ± 5.6	36 ± 6.5	42 ± 9.1	52 ± 16			0.16
	median (min; max)	27 (18; 36)	32 (13; 52)	30 (22; 37)	35 (28; 46)	44 (29; 53)	56 (30; 68)			
MDS-UPDRS total score								< 0.01	Kruskal-Wallis rank sum test	
	mean ± sd	46 ± 11	57 ± 25	58 ± 8.8	67 ± 7.2	82 ± 20	102 ± 29			0.01
	median (min; max)	44 (37; 68)	52 (34; 91)	60 (45; 68)	63 (59; 75)	76 (62; 118)	96 (69; 143)			
NFL								0.03	Kruskal-Wallis rank sum test	
	mean ± sd	18 ± 7	17 ± 5.6	24 ± 6.6	36 ± 14	27 ± 9.1	59 ± 64			0.20
	median (min;	14 (12; 29)	16 (12; 25)	25 (16; 32)	33 (24; 58)	26 (17; 39)	30 (19; 171)			

	max)									
Hyposmia (n)		**22**	**24**	**23**	**21**	**20**				
MDS-UPDRS III								0.27	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	2.7 ± 3.1 1 (0; 10)	4.2 ± 7.1 2 (0; 33)	6.1 ± 8.7 2 (0; 36)	6.5 ± 5.8 7 (0; 17)	7.4 ± 8.6 1.5 (0; 27)				1.0
MDS-UPDRS total score								0.48	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	10 ± 7.2 8 (0; 26)	11 ± 9.8 7 (1; 38)	13 ± 13 8 (0; 45)	14 ± 10 13 (1; 37)	15 ± 12 11 (1; 36)				1.0
NFL								0.95	Kruskal-Wallis rank sum test	
	mean ± sd	15 ± 5.1	16 ± 5.6	16 ± 6.3	25 ± 43	18 ± 9				1.0
	median (min; max)	15 (6.5; 26)	15 (6.3; 32)	14 (7.9; 34)	14 (7.5; 210)	16 (8.7; 46)				
isolated REM Sleep Behaviour Disorder (iRBD; n)		**35**	**34**	**36**	**36**	**32**				
MDS-UPDRS III								0.11	Kruskal-Wallis rank sum test	
	mean ± sd	4.5 ± 4	4.8 ± 4.6	7.6 ± 9.3	8 ± 9.5	11 ± 13				0.45
	median (min; max)	3 (0; 15)	3 (0; 18)	4.5 (0; 41)	6 (0; 49)	7.5 (0; 51)				
MDS-UPDRS total score								0.06	Kruskal-Wallis rank sum test	
	mean ± sd	14 ± 7.2	13 ± 7.6	18 ± 15	20 ± 15	24 ± 21				0.28
	median (min; max)	14 (3; 31)	10 (2; 29)	15 (3; 63)	14 (2; 74)	16 (3; 86)				
NFL								0.25	Kruskal-Wallis rank sum test	
	mean ± sd	17 ± 8	19 ± 14	17 ± 8.3	19 ± 10	18 ± 6.2				0.76
	median (min;	14 (6.8; 39)	16 (6.5; 91)	15 (6.4; 50)	16 (4.9; 49)	17 (6; 34)				

	max)									
PD-GBA (n)		**64**	**63**	**48**	**24**	**2**				
MDS-UPDRS III								0.63	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	23 ± 12 23 (4; 54)	24 ± 12 22 (7; 64)	22 ± 11 20 (4; 51)	21 ± 10 19 (0; 44)	12 ± 11 12 (4; 20)				1.0
MDS-UPDRS total score								0.96	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	40 ± 16 36 (14; 77)	42 ± 18 39 (15; 98)	41 ± 17 38 (13; 85)	40 ± 13 42 (6; 63)	35 ± 20 35 (21; 49)				1.0
NFL								0.65	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	16 ± 11 13 (3.8; 81)	18 ± 13 14 (5; 96)	17 ± 11 14 (4.3; 72)	21 ± 24 14 (7.8; 130)	17 ± 3.5 17 (14; 19)				1.0
PD-LRRK2 (n)		**135**	**126**	**110**	**79**	**70**				
MDS-UPDRS III								0.35	Kruskal-Wallis rank sum test	
	mean ± sd	18 ± 9.4	20 ± 10	18 ± 10	19 ± 9.6	17 ± 8.8				1.0
	median (min; max)	16 (3; 48)	18 (2; 56)	17 (2; 59)	18 (2; 45)	17 (0; 44)				
MDS-UPDRS total score								0.36	Kruskal-Wallis rank sum test	
	mean ± sd median (min; max)	34 ± 16 32 (6; 80)	37 ± 17 34 (8; 113)	33 ± 16 30 (7; 100)	35 ± 16 35 (6; 94)	33 ± 14 32 (6; 70)				1.0
NFL								0.66	Kruskal-Wallis rank sum test	
	mean ± sd	16 ± 9.9	17 ± 13	18 ± 17	48 ± 275	22 ± 57				1.0
	median (min; max)	13 (3.9; 71)	13 (4.4; 90)	14 (5.2; 163)	15 (5; 2463)	14 (6; 486)				
PD-SNCA (n)		**17**	**15**	**17**	**8**	**7**				

MDS-UPDRS III								0.28	Kruskal-Wallis rank sum test	
	mean ± sd	23 ± 20	25 ± 14	24 ± 17	23 ± 14	39 ± 23				0.34
	median (min; max)	14 (3; 71)	23 (7; 52)	17 (4; 65)	20 (6; 51)	29 (16; 80)				
MDS-UPDRS total score								0.10	Kruskal-Wallis rank sum test	
	mean ± sd	42 ± 30	49 ± 26	52 ± 31	52 ± 32	78 ± 39				0.31
	median (min; max)	33 (8; 102)	50 (12; 107)	38 (23; 127)	40 (22; 121)	55 (44; 135)				
NFL								0.17	Kruskal-Wallis rank sum test	
	mean ± sd	13 ± 9.9	20 ± 24	19 ± 13	20 ± 14	21 ± 19				0.34
	median (min; max)	9.7 (5.7; 41)	12 (6.6; 103)	15 (6.3; 54)	14 (9.8; 51)	15 (7.7; 64)				
UN-GBA (n)		**127**	**113**	**95**	**42**					
MDS-UPDRS III								0.01	Kruskal-Wallis rank sum test	
	mean ± sd	2.4 ± 3.7	3.1 ± 4.4	2.9 ± 3.5	4.1 ± 4.3					0.02
	median (min; max)	1 (0; 21)	2 (0; 28)	2 (0; 17)	3 (0; 22)					
MDS-UPDRS total score								0.21	Kruskal-Wallis rank sum test	
	mean ± sd	9.1 ± 7.9	10 ± 8.8	9.8 ± 8.4	12 ± 8.6					0.21
	median (min; max)	7 (0; 44)	8 (0; 50)	8 (0; 47)	9 (0; 36)					
NFL								0.07	Kruskal-Wallis rank sum test	
	mean ± sd	13 ± 7.2	14 ± 7	16 ± 17	15 ± 6.7					0.14
	median (min; max)	12 (3.9; 63)	12 (4.3; 47)	12 (5.6; 168)	15 (4.3; 36)					
UN-LRRK2 (n)		**166**	**141**	**137**	**98**	**52**				
MDS-UPDRS III								0.49	Kruskal-Wallis rank sum test	

	mean ± sd	3 ± 4.1	3.3 ± 4.3	3.6 ± 4.6	3.3 ± 4.7	3.8 ± 4.2				1.0
	median (min; max)	2 (0; 27)	2 (0; 21)	2 (0; 27)	2 (0; 25)	4 (0; 19)				
MDS-UPDRS total score								0.67	Kruskal-Wallis rank sum test	
	mean ± sd	8.5 ± 7.7	8.7 ± 7.3	9.7 ± 8.8	9.4 ± 11	9.1 ± 7				1.0
	median (min; max)	6.5 (0; 45)	7 (0; 42)	8 (0; 47)	6 (0; 64)	8 (0; 27)				
NFL								0.84	Kruskal-Wallis rank sum test	
	mean ± sd	13 ± 6.2	14 ± 8.6	13 ± 6.7	13 ± 5.6	14 ± 7.1				1.0
	median (min; max)	11 (2.3; 47)	12 (3.6; 88)	12 (3.2; 50)	12 (3.3; 30)	11 (3.2; 41)				
UN-SNCA (n)		**5**	**5**	**5**	**3**	**3**				
MDS-UPDRS III								NaN	Kruskal-Wallis rank sum test	
	mean ± sd	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0				NaN.0
	median (min; max)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)				
MDS-UPDRS total score								0.90	Kruskal-Wallis rank sum test	
	mean ± sd	3.4 ± 2.7	2.6 ± 2.1	4 ± 2	3 ± 2	4.3 ± 4.9				1.0
	median (min; max)	3 (1; 8)	3 (0; 5)	4 (2; 6)	3 (1; 5)	2 (1; 10)				
NFL								0.93	Kruskal-Wallis rank sum test	
	mean ± sd	5.7 ± 3.4	6.2 ± 3.5	5.6 ± 2.1	8.2 ± 4.8	6.2 ± 3.2				1.0
	median (min; max)	5.3 (2; 11)	5.2 (3.2; 12)	5.5 (3.3; 8.6)	6.6 (4.3; 14)	5.8 (3.2; 9.5)				

	Estimate	**Standard Error**	**p-value**
Intercept	0·0389	0·0224	0·0829
Baseline Log10(NfL)	0·763	0·0192	**<0·0001**
Baseline Age (years)	0·0034	0·0004	**<0·0001**

Gender (female)	0·0178	0·0079	**0·0235**
Time (years)	0.0141	0.0027	**<0.0001**
Genetic Cohort-Unaffected	0·0078	0·0133	0·5574
Prodromal condition	0·0213	0·0208	0·3077
Parkinson's Disease	0·0075	0·0113	0·5116
Genetic Cohort-PD	0·0468	0·0135	**0·0006**
Other Neurodegenerative Diseases	0·109	0·0461	**0·0180**
Time: Female	-0·0025	0·0024	0·3046
Time: Genetic Cohort-Unaffected	-0·0026	0·0064	0·6874
Time: Prodromal	-0·00004	0·0082	0·9962
Time: Parkinson's Disease	0·0154	0·0029	**<0·0001**
Time: Genetic Cohort-PD	0·0017	0·0054	0·7558
Time: Other Neurodegenerative Diseases	0·0552	0·0115	**<0·0001**

*Healthy controls and male are the references of the categorical variables.

Abbreviations: Neurofilament light chain (NfL)

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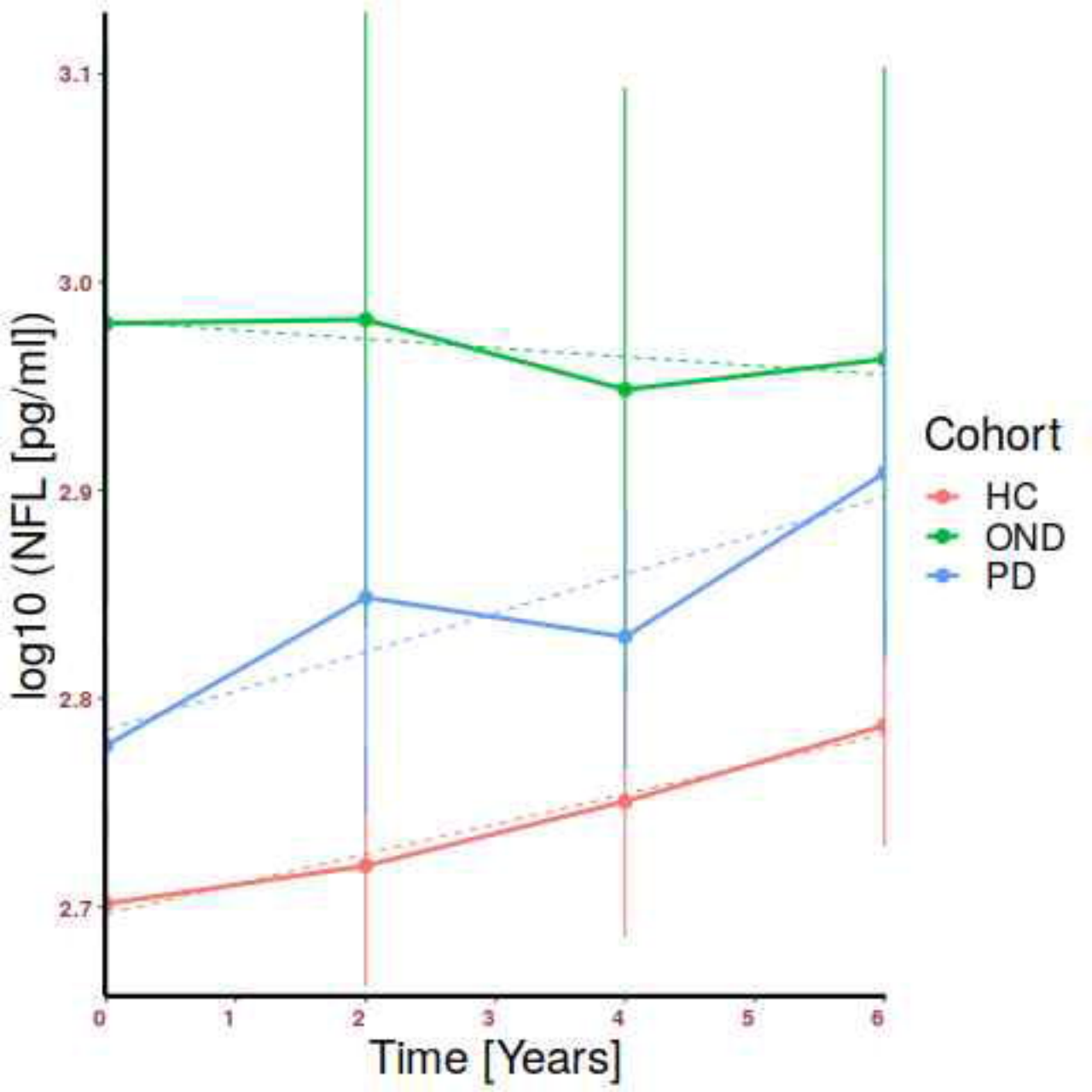
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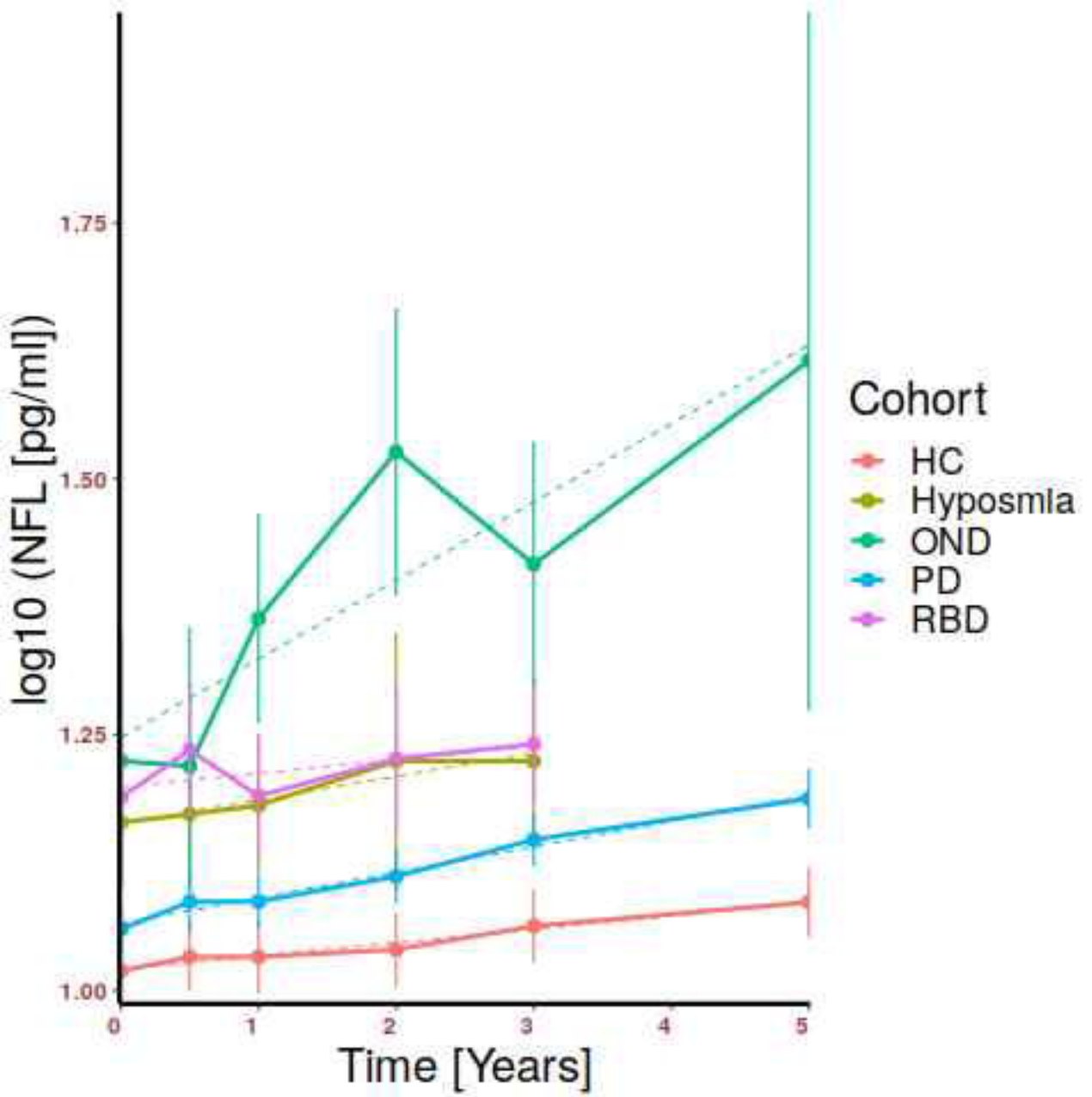
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1.50
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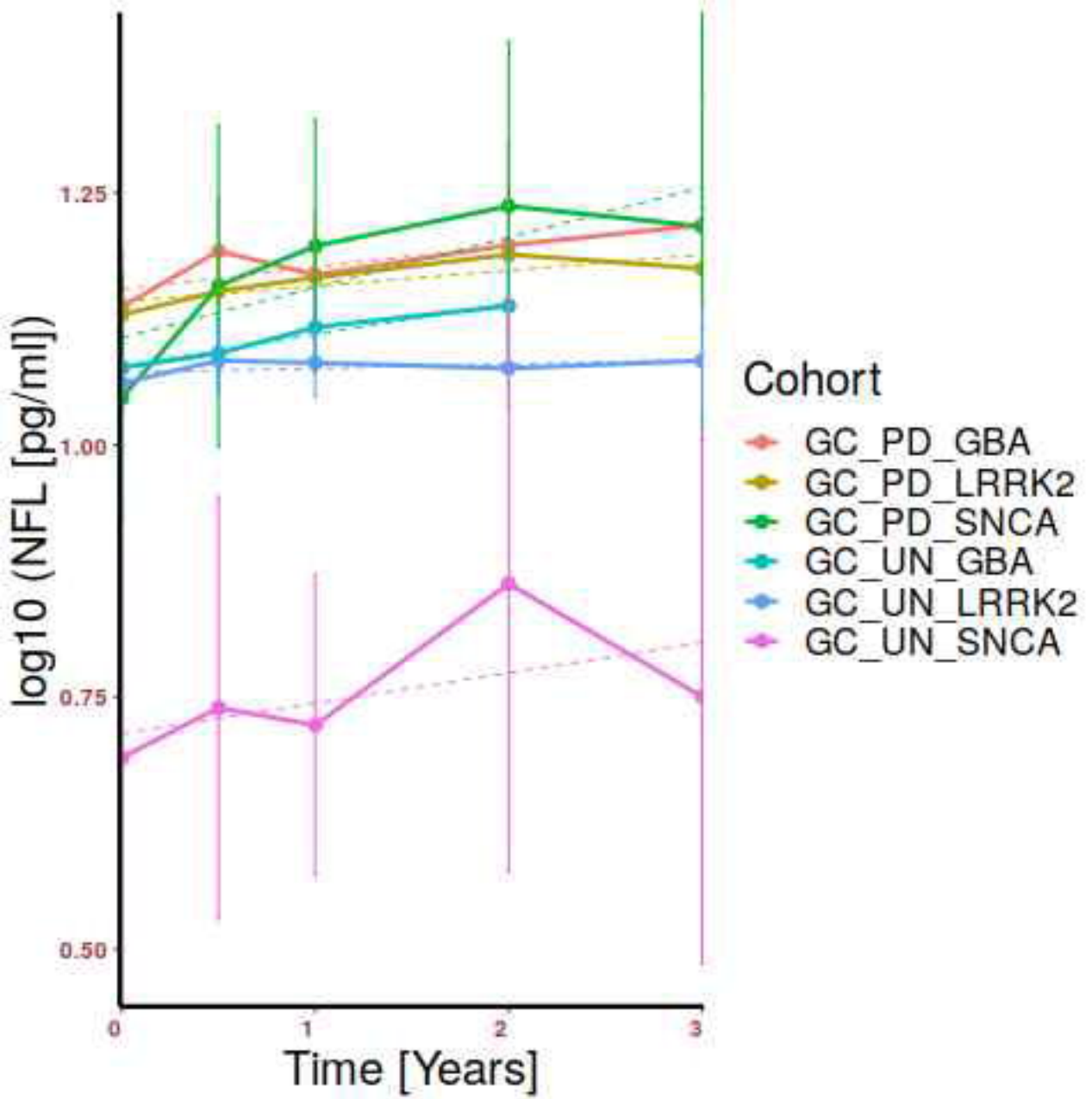
4
5
Time [Years]
Cohort
HC
Hyposmia
OND
PD
RBD


log10 (NFL [pg/ml])
1.25
1.00
0.75
0.50
0
1
2
3
Time [Years]
Cohort
GC_PD_GBA
GC_PD_LRRK2
GC_PD_SNCA
GC_UN_GBA
GC_UN_LRRK2
GC_UN_SNCA