BRIEF REPORT

Intraindividual Neurofilament Dynamics in Serum Mark the Conversion to Sporadic Parkinson's Disease

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ABSTRACT: Background and Objectives: With disease-modifying treatment strategies on the horizon, stratification of individual patients at the earliest stages of Parkinson's disease (PD) is key—ideally already at clinical disease onset. Blood levels of neurofilament light chain (NfL) provide an easily accessible fluid biomarker that might allow capturing the conversion from prodromal to manifest PD.

Methods: We assessed longitudinal serum NfL levels in subjects converting from prodromal to manifest sporadic PD (converters), at-risk subjects, and matched controls (72 participants with ≈4 visits), using single-molecule array (Simoa) technique.

Results: While NfL levels were not increased at the prodromal stage, subjects converting to the manifest motor stage showed a significant intraindividual acceleration of the age-dependent increase of NfL levels.

Conclusions: The temporal dynamics of intraindividual NfL blood levels might mark the conversion to clinically manifest PD, providing a potential stratification biomarker for individual disease onset in the advent of precision medicine for PD. © 2020 The Authors. *Movement Disorders* published by Wiley Periodicals, Inc. on behalf of International Parkinson and Movement Disorder Society.

Key Words: biomarker; longitudinal study; neurofilament light chain (NfL); Parkinson's disease (PD); premanifest disease; prodromal symptoms; serum; single molecule array (Simoa) technique

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A precision medicine approach to Parkinson's disease (PD) requires the stratification of patients with PD into well-defined subgroups that might be amenable to stage-specific and risk-specific disease-modifying therapies.[1] Easily accessible fluid biomarkers supporting this stratification would present an important step towards a precision medicine approach to PD. Such biomarkers should particularly allow delineating the earliest disease stages of PD, ideally also the conversion from prodromal to clinically established PD, where future disease-modifying therapies could likely have the greatest therapeutic effects.[2-4] Blood levels of neurofilament light chain (NfL), a neuronal cytoskeletal protein released upon neuronal damage, might provide a promising candidate biomarker as elevated NfL levels have been reported in the course of clinically established PD[5-9] and associated with more rapid individual disease progression in PD, both in terms of motor and cognitive functions.[5,6]

While there is thus increasing evidence to support the utility of NfL levels during the clinically established stage of PD, we hypothesized that blood levels of NfL in PD start increasing in individual subjects already during the period of conversion from prodromal to clinically established disease. To test this hypothesis, we longitudinally assessed the serum levels of NfL in individuals converting from prodromal to clinically established PD (converters)—with samples taken both before and after the diagnosis of clinically established PD—and compared them with those of healthy age-matched controls who remained without any risk markers of prodromal PD (controls). To scrutinize the specificity of our findings in the converter group, we assessed 2 additional groups with an increased risk of prodromal PD but without conversion to manifest PD: individuals accumulating an increasing number of risk markers of prodromal PD during the study (progressors, high likelihood of conversion, but not yet quantified conversion rate)[10,11] and individuals with probable rapid eye movement sleep behavior disorder (RBD subjects, conversion rate ≈6% per year).[12]

Methods

Participants

All participants were prospectively assessed as part of the Tübingen Evaluation of Risk Factors for Early Detection of Neurodegeneration (TREND) study.[13] The TREND study recruited 1201 participants aged 50 to 80 years in the period from 2009 to 2014, with follow-up assessments every 2 years for clinical signs of neurodegenerative disease and risk factors of prodromal PD, specifically depression, hyposmia, probable RBD, and hyperechogenicity of the substantia nigra on transcranial ultrasound. Participant recruitment, phenotyping, and grouping are described in detail in Supplement 1. In brief, for the present investigation (72 participants, 324 visits), the group of converters (n = 16) comprised all TREND participants who developed PD during the prospective follow-up as defined by UK Brain Bank criteria. For healthy controls (n = 20), we randomly selected age-matched and sex-matched neurologically healthy participants who remained without any signs of neurodegenerative disease and without any risk factors of prodromal PD throughout the study. The group of progressors (n = 20) was composed of participants who showed at least 1 factor of prodromal PD at their baseline visit and accumulated further persistent prodromal factors during the study. The group of RBD participants (n = 16) was composed of individuals with probable RBD[14] already present at their baseline visit and confirmed at every follow-up visit. The groups did not differ significantly in age, sex, or number of follow-up visits (Supplement 2). All individuals provided written informed consent prior to participation according to the Declaration of Helsinki. The ethics committee of the University of Tübingen approved the study (90/2009BO2) and the analysis (480/2015BO2).

Biomaterial

Blood samples were centrifuged (4000*g*, 10 minutes). Serum was frozen at −80°C within 60 minutes after collection and stored and analyzed without any previous thaw–freeze cycle.

NfL Measurement

Serum NfL levels were measured in duplicates by single-molecule array (Simoa) technique on the Simoa HD-1 Analyzer (Quanterix, Lexington, MA) as established previously.[15] Technicians were blinded to the participants' status.

Analysis

We analyzed NfL levels (log transformed) with a linear mixed effects model with the fixed factors group (converters, healthy controls, progressors, RBD subjects) and age (centred at the mean age of all participant visits, ie, 70 years), their interaction, and the random variable participant, modeled by random intercepts. The addition of random slopes did not improve model fit. We analyzed the fixed effect of group and the interaction of group and age with contrasts (Bonferroni-corrected). Model estimates for each group were visualized for the absolute NfL concentrations (back transformed) at 70 years of age and for the annual NfL increase (reported as estimated mean and 95% confidence interval; Fig. 1). As linear increases of the log-transformed NfL levels correspond to exponential increases of original NfL levels over age, annual increases of NfL levels were expressed as the percentage of annual increase. We analyzed and visualized the data in R (*R Foundation for Statistical Computing*, Vienna, Austria) using the packages

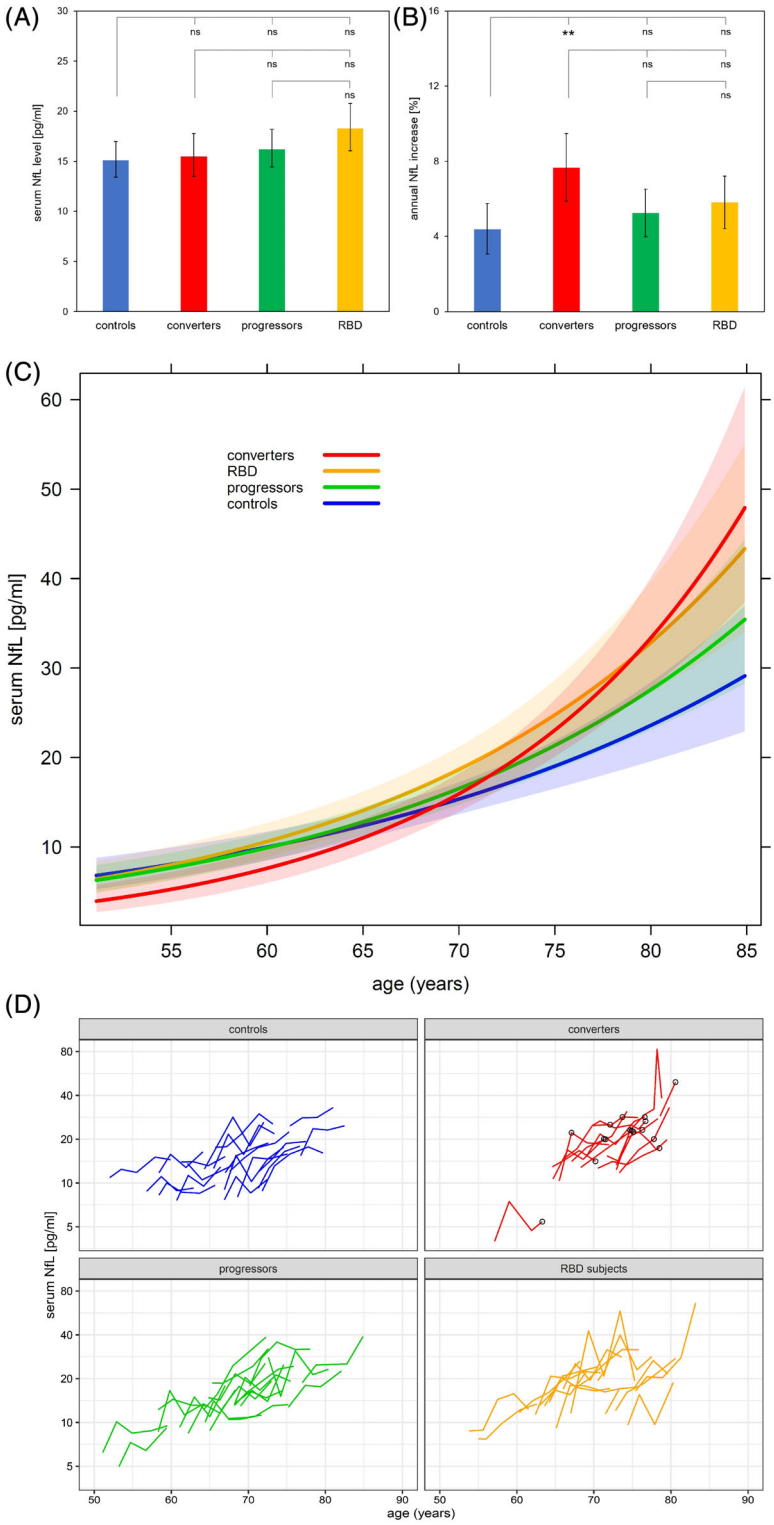

FIG. 1. Temporal dynamics of serum NfL levels in individuals developing Parkinson's disease and age-matched control groups. (**A**,**B**) NfL levels (log transformed) were analyzed with a linear mixed effects model with the fixed factors group (converters, controls, progressors, RBD subjects) and age, their interaction, and the random variable subject. Bar plots display the absolute levels of NfL (pg/ml; at 70 years of age) and their annual increases (%), as estimated by the model (mean and 95% confidence interval). (**C**) Model estimates of the temporal NfL dynamics are displayed for each group (solid line, mean; shaded area, 95% confidence interval). (**D**) The original NfL serum concentrations are plotted over age, with each line connecting the longitudinal measurements of a single subject. In converters, the circle highlights the first visit in which clinically established PD was present. NfL, neurofilament light chain; ns, nonsignificant; RBD, rapid eye movement sleep behavior disorder. $^{**}P < .01$, ns $P \geq 0.05$, Bonferroni corrected. [Color figure can be viewed at wileyonlinelibrary.com]

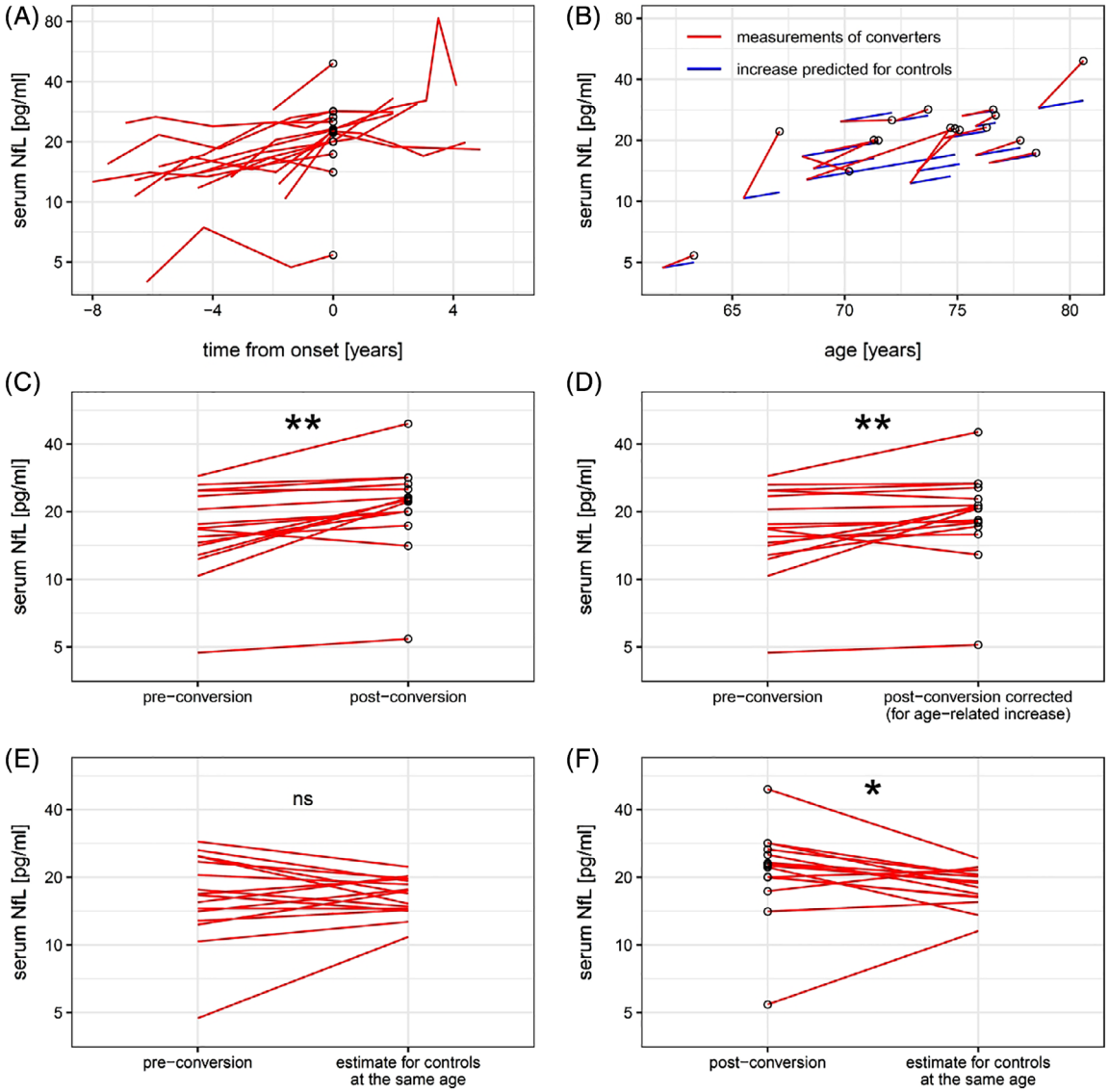

FIG. 2. NfL levels before and after conversion to clinically established Parkinson's disease. (**A**) Longitudinal NfL levels of converters (n = 16) are plotted over the time from the individual onset of clinically established Parkinson's disease (visit marked by black circle). (**B**) For each converter, preconversion and postconversion NfL levels are plotted over age, connected by a red line. The black circle marks the postconversion visit. The blue line shows the age-related increase that one would expect in controls during the time interval from the preconversion to the postconversion visit (4.4% per year). (**C**) Individual NfL levels increase significantly from the preconversion to the postconversion visit. (**D**) The increase in converters remains significant if corrected for the age-related increase one would expect in controls during the interval from the preconversion to the postconversion visit. (**E**) NfL levels at the preconversion visit were not significantly higher than the levels estimated for controls at the same age. (**F**) NfL levels at the postconversion visit were significantly higher than the levels estimated for controls at the same age. Wilcoxon tests, 2-sided, $^{**}P < 0.01$, $^{*}P < 0.05$, ns: $P \geq 0.05$. Note the logarithmic scale of the *y* axis. NfL, neurofilament light chain; ns, nonsignificant. [Color figure can be viewed at wileyonlinelibrary.com]

lme4, multcomp, lsmeans, and effects. We identified and excluded 1 outlying NfL value (in the progressor group) based on its leverage and Cook's distance. The assumption of normality was tenable for the remaining data.

Results

Absolute Levels of Serum NfL

The mixed effects model revealed that NfL levels in serum did not differ significantly between the 4 groups ($P = 0.161$; Fig. 1A), but significantly increased with age ($P < 0.001$). The estimated NfL level at 70 years of age was 15.1 pg/ml (13.4–17.0) in controls, 15.5 pg/ml (13.5–17.8) in converters, 16.2 pg/ml (14.4–18.2) in progressors, and 18.3 pg/ml (16.0–20.8) in RBD subjects (estimated mean and 95% confidence interval).

Annual Increase of NfL Levels

There was a significant interaction between the factors age and group ($P = 0.037$), indicating that the

annual increase of NfL levels differed between the groups (Fig. 1B). Contrasts revealed a significant difference in the annual increase of NfL levels between converters (7.7% [5.9–9.5]) and controls (4.4% [3.0–5.7]; $P = 0.009$, 2-sided, Bonferroni corrected). This effect was confirmed as a trend if the analysis was limited to the symptomatic disease stage of converters ($P = 0.065$) but lost in their presymptomatic stage ($P = 0.456$). The annual increase in converters was also significantly higher than in all 3 other groups pooled together ($P = 0.011$), but did not differ significantly from progressors ($P = 0.062$) and from RBD subjects ($P = 0.222$) when considered singularly (Fig. 1B). The annual NfL increase was not significantly elevated in progressors (5.2% [4.0–6.5]) and RBD subjects (5.8% [4.4–7.2]) when compared with controls (both $P \geq 0.100$).

NfL Levels Before and After Conversion to Clinically Established PD

Converters (n = 16) showed a significant intraindividual increase of NfL levels from the preconversion visit to the postconversion visit ($P = 0.001$, Wilcoxon test, 2-sided; Fig. 2C). This increase remained significant ($P = 0.008$) if corrected for the age-related increase that one would expect in controls during the time interval from the preconversion to the postconversion visit (Fig. 2D). We based the correction on the annual increase rate in controls, as estimated by the mixed effects model (ie, 4.4% per year). While NfL levels at the preconversion visit were not significantly higher than the NfL levels predicted for controls at the same age ($P = 0.860$; Fig. 2E), the NfL levels at the postconversion visit were significantly increased when compared with controls at the same age ($P = 0.039$; Fig. 2F). However, NfL levels at the postconversion visit were not significantly increased when compared with the levels predicted for progressors ($P = 0.298$) and RBD subjects ($P = 0.323$) at the same age.

NfL and Probability of Prodromal PD

NfL levels were not significantly associated with the participants' probability of having prodromal PD,[16] as revealed by a mixed model with the factors probability of prodromal PD ($P = 0.271$), age ($P < 0.001$), and their interaction ($P = 0.966$; Supplement 3). In progressors, the appearance of the novel prodromal factor was associated with an intraindividual increase of NfL levels that did not exceed the annual increase expected for controls in the same time interval (Supplement 4).

NfL Levels and Cognition

The participants' cognitive function, assessed by the Mini Mental Status Exam, did not differ significantly between groups and was not significantly associated with NfL levels (Supplement 5).

Discussion

Our prospective, longitudinal study of NfL blood levels in prodromal PD demonstrates that the intraindividual levels of NfL start increasing at the time of conversion from prodromal to clinically established PD. It shows for the first time that the concept of increasing NfL levels in proximity to phenoconversion is applicable not only to autosomal-dominant neurodegenerative model diseases[17-19] but also to sporadic neurodegenerative diseases.

While previous studies indicated some degree of increased NfL levels in clinically established PD as assessed several years after conversion,[5-9] our study locates the timing of the increase of NfL blood levels in sporadic PD to the conversion period from the prodromal stage to the clinically established stage of PD. This notion is evidenced by the NfL increase in the converter group, which was present at the postconversion visit, but still absent at the preconversion visit, and supported by the absence of NfL increases in nonconverting participants with an increased risk of converting to clinically established PD, that is, RBD subjects and subjects accumulating an increasing number of risk factors of prodromal PD. It was further corroborated by the missing correlation of NfL levels with the estimated quantitative risk of having prodromal PD. Unlike in more rapidly progressive neurodegenerative diseases, such as frontotemporal dementia, amyotrophic lateral sclerosis, and repeat-expansion spinocerebellar ataxias,[19-21] the NfL increase in sporadic PD thus does not appear to precede phenoconversion but, rather, occurs in its close proximity. Although the absence of a significant NfL increase before conversion might be disappointing at first glance, we expect our findings from a comprehensively characterized cohort with a relevant number of converters to be valuable for defining and further evaluating the prodromal stage of PD: stable NfL levels at the prodromal stage might be used to indicate stable disease and low risk of imminent conversion, whereas accelerated NfL increases might be used to capture the conversion to the clinically established stage.

The annual increase rate of NfL levels in converters was accelerated in the conversion period, whereas the absolute levels during this period did not yet differ from those of age-matched healthy controls. Thus, the temporal dynamics of NfL levels within individual PD individuals might be more sensitive for detecting neuronal decay than the absolute levels, as already also suggested for autosomal-dominant diseases.[18]

Although the increase in the intraindividual NfL dynamics was statistically significant, the effect was quantitatively small. This finding agrees with the notion that NfL provides a sensitive marker of axonal decay that is not typically largely increased in the earliest stages of PD.[2] In contrast to PD, NfL levels are much higher in atypical parkinsonian disorders, which show

more widespread and faster neurodegeneration, involving also higher rates of axonal decay.[7,22-25]

In sum, although our findings do not support the use of blood NfL as biomarker of risk stratification in the prodromal stage of sporadic PD, they delineate the temporal onset of the blood NfL increase in PD and suggest NfL as a blood biomarker capturing the conversion to the clinically established stage. Thus, our results help define the disease stage in which blood NfL levels might fulfil their suggested role as biomarker of disease progression, paving the way toward precision medicine approaches for PD.

Data Availability

The datasets analyzed in the current study are available from the corresponding author on reasonable request.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.