Association of the Level of Neurofilament Light With Disease Severity in Patients With Spinocerebellar Ataxia Type 2

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Abstract

Background and Objectives: Few biochemical markers have been identified in spinocerebellar ataxia type 2 (SCA2). This study aimed to determine the levels of neurofilament light (NfL) in patients with SCA2 and identify whether they were associated with disease severity.

Methods: Participants were recruited from one medical center in China, and individuals with SCA2 were genetically diagnosed. NfL levels were assessed using the single molecule array method. Disease severity was evaluated using the Scale for the Assessment and Rating of Ataxia (SARA), the International Cooperative Ataxia Rating Scale (ICARS), and the Inventory of Non-Ataxia Symptoms (INAS). Cerebellum and brainstem volumes were calculated using neuroimaging measurements. We used Pearson's correlation and partial correlation for correlation analyses.

Results: Forty-nine manifest patients with SCA2, 10 preclinical individuals with SCA2 and 92 controls were enrolled. A high consistency was identified between serum and CSF NfL ($r = 0.868, p < 0.0001$). In individuals with SCA2, levels of serum NfL were associated with disease severity (SARA, $r = 0.425, p = 0.003$; ICARS, $r = 0.383, p = 0.009$; INAS, $r = 0.390, p = 0.007$; cerebellum volume, $r = -0.393, p = 0.024$) after
adjustment for age. NfL levels were higher close to the expected age of onset in preclinical individuals with SCA2 ($R^2 = 0.43$, $p = 0.04$).

**Discussion:** Levels of serum NfL were correlated with disease intensity in individuals with SCA2, and were higher close to the estimated age of onset in preclinical SCA2. Therefore, NfL is a potential serum biomarker of disease severity in SCA2.

**Classification of Evidence:** This study provides Class II evidence that elevated NfL levels are associated with disease severity in individuals with SCA2.

**Introduction**
Spinocerebellar ataxia type 2 (SCA2) is one of the most common autosomal dominantly inherited degenerative ataxias worldwide, clinically characterized by progressive ataxia, slow hypometric saccades, hyporeflexia, and polyneuropathy. Considering the upcoming disease-modifying treatments and several promising therapies, accessible, reliable and objective tools are immediately needed, to stratify individuals with SCA2 with different stages, evaluate disease severity, track disease progression and further pave the way for future trials. Although extensive efforts have established neuroimaging and electrophysiology biomarkers of progression, few biochemical markers have been identified.

Neurofilament light (NfL) chain is gaining increasing attention as a candidate biomarker because the release of NfL sharply increases in response to axonal damage. Recent studies have validated NfL as a promising biomarker in many neurodegenerative diseases, including parkinsonian disorders, Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and dementia. We have reported that NfL was higher in CSF and serum in patients with spinocerebellar ataxia type 3 (SCA3) and correlated with clinical severity. However, a previous work observed no obvious elevation of NfL level in two patients with SCA2. Thus, there is a
compelling need to determine whether NfL could act as a potential biomarker of neurodegeneration in a larger cohort.

In this study, we aimed to investigate the levels of serum and CSF NfL in individuals with SCA2 with different disease stages, and to identify whether NfL is associated with disease severity.

Methods

Standard protocol approvals, registrations, and patient consents
The study was approved by the ethics committee of the Second Affiliated Hospital, Zhejiang University School of Medicine. The written informed consent was obtained from each participant before recruitment.

Primary research questions/classification of evidence
Our primary research question is to determine the levels of NfL in individuals with SCA2 and identify whether they are associated with disease severity. This study was designed to provide Class II evidence that elevated NfL levels were associated with clinical disease severity in individuals with SCA2.

Participants and study design
In the cross-sectional study, 92 controls and 59 individuals with SCA2 including 10 preclinical individuals (preSCA2) and 49 manifest patients were recruited from April 2015 to September 2020 in the Second Affiliated Hospital of Zhejiang University School of Medicine (Hangzhou). These 59 individuals with SCA2 were genetically diagnosed as previously reported. The unrelated neurologically healthy controls were with negative genetic screening for ATXN2 gene and had no evidence of inherited disease, neurological and psychiatric diseases. Age and sex were considered in the control selection. Demographic data, clinical characteristics, rating scales and brain magnetic resonance imaging (MRI) information were collected from individuals with SCA2. Age was defined as the age at sample collection. The
preclinical stage was defined by unspecific neurological symptoms (e.g., muscle cramps, vertigo, hyporeflexia) and/or mild coordination deficit with the Scale for the Assessment and Rating of Ataxia (SARA) \(^{20}\) score < 3 according to the reported criteria \(^{21}\). The manifest SCA2 were classified into two subgroups based on median SARA scores and clinical features, including 25 Stage 1 (3 ≤ SARA < 11) and 24 Stage 2 (SARA ≥ 11) patients.

Blood and CSF samples were collected from participants. Forty-three individuals consisting of 23 manifest patients with SCA2 and 20 controls were classified into Group A due to the availability of both CSF and serum to assess the correlation of the NfL level between them. All participants with available serum sample including 92 controls and 59 individuals with SCA2, among which 23 manifest patients and 20 controls were from Group A, were classified into Group B for further study.

**Clinical scales and neuroimaging assessments**

Three rating scales, including SARA, the International Cooperative Ataxia Rating Scale (ICARS) \(^{22}\), and the Inventory of Non-Ataxia Symptoms (INAS) \(^{23}\) were available in the same 47 individuals, to assess disease severity. SARA score ranges from 0 to 40 with 0 indicating no ataxia and 40 the most severe degree of ataxia. The 100-point ICARS comprises 19 items with 0 suggesting absence of ataxia and 100 the most severe degree of ataxia. INAS count ranges from 0 to 16 to evaluate non-ataxia signs. The rating scale assessments were performed by two experienced neurologists who were blinded to the NfL levels of participants, within 7 days after sample collection.

T1-weighted MRI scans were performed on a subgroup of 34 manifest patients with SCA2 (Signa HDx, General Electric Healthcare, Chicago, IL, USA) within 7 days after sample collection, with the following parameters: repetition time = 1750ms, echo time = 14ms, field of view = 24cm × 24cm, inversion time = 720ms, and slice...
thickness = 6mm. All scans passed visual quality control check for artifacts before processing. Cerebellum, brainstem and total intracranial regions of each scan were segmented by manual identification to ensure accuracy, and calculated by ITK-SNAP software (Version 3.8) to obtain the volumes \(^{24}\). The analyses of MRI data were carried out by investigators unaware of disease status. The cerebellum and brainstem volumes were expressed as percentages of total intracranial volumes.

**Serum and CSF NfL quantification**

For each participant, peripheral blood was drawn by venipuncture from median cubital vein under fasting condition lasting approximately 10 hours, and collected in BD Vacutainer tubes (Plymouth PL6 7BP, UK). Blood samples were rested for 30 to 60 minutes at room temperature before centrifugation at 2,000 g for 10 minutes. Then the supernatant was divided into aliquots and stored in Protein LoBind Tubes (Eppendorf AG, Germany) at -80°C immediately. These procedures of serum collection were completed within 2 hours. CSF samples were collected by lumbar puncture at vertebral body L3-5 using atraumatic needles. The first 1-2 mL of CSF was discarded and the next 12-15 mL was collected, and was immediately placed at 4°C. CSF was centrifuged at 400 g for 10 minutes to remove cells within a maximum interval of 10 minutes after collection, and then was divided into aliquots, and immediately stored in Protein LoBind Tubes at -80°C. The serum and CSF samples were collected according to the recommended guideline \(^{25}\) and were sent to analysis without any thaw-freeze cycle. The NfL was quantified by ultra-sensitive single-molecule array (Simoa) technique on the Simoa HD-X Analyzer (Quanterix, MA, US) at GBIO (Hangzhou, China) using Simoa NF-light Advantage Kit (Product Number: 103186). Serum and CSF samples were diluted at ratios of 1:4 and 1:100, respectively. One third of samples were detected in duplicates and the intra- and inter-assay variabilities were below 10%. All NfL values were within the linear ranges.
of the assay. Operators were blinded to participants’ disease status.

**Statistical analysis**

Demographic and clinical data were described as mean (standard deviation) if normally distributed, or median (interquartile range) if non-normally distributed unless otherwise specified. Shapiro-Wilk test was used to test the normality. NfL levels in serum and CSF were non-normally distributed because of biologically higher values. Natural log-transformation produced acceptable normal distribution for both serum and CSF NfL, and was conducted for subsequent analyses if appropriate. Potentially confounding variables (age, sex, normal and expanded CAG repeat length, and disease duration) were preliminarily analyzed, and those with significant effect were included as covariates for subsequent analyses. To assess intergroup differences of demographics and clinical characteristics, unpaired two-sample \( t \) test or one-way analysis of variance was used on the premise of normality and homogeneity, otherwise nonparametric Mann-Whitney \( U \) test was used. Intergroup NfL level comparisons were conducted using multiple linear regressions including age and expanded CAG repeat length as covariates.

Generalized estimating equations (GEEs) were utilized to examine influence of familiarity on NfL levels, and repeatedly compare intergroup NfL levels while controlling for clustering of individuals with SCA2 within a family. Repeated measures on individuals within a family were included with an independent working correlation matrix. Correlation analyses were performed using Pearson’s correlation and partial correlation for age adjustment. Spearman rank correlation was used for variables if appropriate. Residuals from the regression of NfL level or rating scale scores or affected brain volumes on age represented the component of those dependent variables not explained by age. Age-adjusted values were obtained by adding residuals to the mean NfL level or rating scale scores or affected brain volumes.
To evaluate the diagnostic power of NfL, we used receiver operating characteristic (ROC) curves and compared area under the curve (AUC) as suggested by previous study. Analyses were performed with SPSS (IBM, Version 22). Significant level was defined as $p < 0.05$. After a Bonferroni correction, 0.05 is divided by the number of performed statistical tests and a $p$ value $< 0.017$ was considered as statistically significant.

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Results**

**Preliminary analyses of potentially confounding variables for NfL**

The demographics and clinical characteristics of Group A and Group B are summarized in Table 1. Primary data in detail was provided in eTable 1 and eTable 2. We assessed potential confounding variables for NfL levels in CSF and serum (Figure 1). In controls, NfL levels in both CSF and serum were associated with age ($r = 0.607$, $p = 0.005$; $r = 0.620$, $p < 0.0001$). No evidence for effect of sex on NfL level was observed in CSF and serum ($p = 0.650$, $p = 0.698$). In individuals with SCA2, NfL levels in serum were moderately associated with expanded CAG repeat length ($r = 0.446$, $p = 0.0004$) while those in CSF not ($p = 0.408$). Age, sex, disease duration and normal CAG repeat length were associated with NfL levels in neither CSF nor serum (eTable 3). As the 59 individuals with SCA2 were from 46 families, a GEE model was conducted to explore how much genetic and environmental backgrounds accounting for the variation of NfL level. It seemed that no obvious differences were found in the NfL levels between one SCA2 individual and either affected parent or sibling ($p = 0.053$, $p = 0.27$). In Group A, there was no difference in age between the controls and manifest patients with SCA2 ($p = 0.13$). In Group B, the controls were recruited to
match the mean age of manifest patients. The preSCA2 individuals were younger than the controls and manifest patients with SCA2 ($p = 0.01$ and $p = 0.01$, respectively), a consequence of the condition that preSCA2 individuals were too young to have developed ataxia. Thus, age adjustment was performed in all analyses to minimize the effect of age. As expanded CAG repeat is the primary driver and the known best predictor of disease progression\textsuperscript{1}, we also included expanded CAG repeat length as covariate in the intergroup comparison of NfL levels, to explore the independent discriminatory ability of NfL.

**A high consistency was identified between serum and CSF NfL of patients with SCA2**

To assess the association of NfL between serum and CSF, a Group A of 23 manifest patients with SCA2 and 20 controls was firstly studied. The median levels of NfL in CSF and matched serum were respectively 6.07 and 4.05 times higher in manifest patients with SCA2 than in controls (CSF, 2187.86 pg/mL vs. 360.50 pg/mL; serum, 27.61 pg/mL vs. 6.81 pg/mL), and the difference remained significant after age adjustment (both $p < 0.0001$; Figure 2A-D). In accord with CNS origin, the median NfL level was 59.85 times higher in CSF than in serum (1113.72 pg/mL vs. 18.61 pg/mL). The ratio further differed between manifest patients and controls (median, 83.21 vs. 52.29, $p = 0.004$). Moreover, there was an association between CSF and serum NfL levels ($r = 0.868$, $p < 0.0001$; Figure 2E), indicating a very high degree of consistency. Furthermore, we compared the discriminatory power of CSF and serum NfL, using ROC curves and the AUC (Figure 2F). For distinguishing between manifest patients and controls, CSF NfL had essentially perfect accuracy (AUC, 1). Matched serum NfL also exhibited excellent accuracy [AUC, 0.987 (95% CI, 0.962 to 1)].

**Serum NfL was elevated in individuals with SCA2**
In a larger serum group (Group B) consisting of 59 individuals with SCA2 and 92 controls, the level of serum NfL was higher in individuals with SCA2 compared to controls after age adjustment using a multiple linear regression model ($p < 0.0001$; median, 23.36 pg/mL vs. 6.79 pg/mL). As shown in Figure 3A, level of serum NfL was remarkably elevated in manifest patients than preSCA2 ($p < 0.0001$, Bonferroni-corrected threshold = 0.017; median, 26.07 pg/mL vs. 15.56 pg/mL). The significance existed after Bonferroni correction. The level of serum NfL was also higher in preSCA2 than in controls ($p < 0.0001$, Bonferroni-corrected threshold = 0.017; median, 15.56 pg/mL vs. 6.79 pg/mL), surviving rigorous Bonferroni correction. And the difference remained significant after age adjustment (Figure 3B). After adjustment for age and expanded CAG repeat length, the manifest SCA2 subgroup had a higher level of serum NfL compared to the preSCA2 subgroup ($p = 0.007$). Likewise, a GEE model adjusting for familiarity and age revealed NfL levels between manifest patients and preSCA2, between manifest patients and controls, and between preSCA2 and controls were different ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$).

For distinguishing between controls and individuals with SCA2, serum NfL had high accuracy [AUC, 0.945 (95% CI, 0.906 to 0.984); Figure 3C]. It exhibited great accuracy to discriminate between manifest patients and controls [AUC, 0.973 (95% CI, 0.947 to 1)] as well. Serum NfL showed moderate accuracy to distinguish manifest patients from preSCA2 with an AUC of 0.847 (95% CI, 0.724 to 0.970), and preSCA2 from controls with an AUC of 0.811 (95% CI, 0.660 to 0.962). Additionally, a level of 14.5 pg/mL as the cutoff value displayed a good sensitivity and specificity for SCA2 detection (88% and 95%, respectively).

**Serum NfL was associated with disease severity**

In the 47 SCA2 individuals with rating scales (8 individuals at preclinical stage, 19 individuals at stage 1, and 20 individuals at stage 2), levels of serum NfL were
associated with SARA ($r = 0.412, p = 0.004$), ICARS ($r = 0.368, p = 0.011$), and INAS scores ($r = 0.360, p = 0.013$). And the correlations remained significant after adjustment for age (SARA, $r = 0.425, p = 0.003$, Figure 4A; ICARS, $r = 0.383, p = 0.009$, Figure 4B; INAS, $r = 0.390, p = 0.007$, Figure 4C). In the subgroup of 34 manifest patients with a brain MRI scan (16 individuals at stage 1, and 18 individuals at stage 2), we observed negative correlation between level of serum NfL and cerebellum volume ($r = -0.555, p = 0.001$), expressed as percentage of intracranial volume. The correlation survived adjustment for age ($r = -0.393, p = 0.024$, Figure 4D). No significant correlation was observed between level of serum NfL and brainstem volume. Therefore, higher level of serum NfL was associated with more severe disease condition and smaller cerebellar volume. A post-hoc analysis showed a sample size of 34 would achieve 86% power to detect a correlation ($r = 0.45$) between levels of NfL in serum and cerebellum volumes using a one-sided hypothesis with a significance level of 5%.

**Higher serum NfL levels indicated proximity to the estimated age of onset in preclinical SCA2**

For 10 individuals at preclinical stage, nystagmus was detected in four individuals, reduced reflexes in two, abnormal finger-to-nose test in one, abnormal heel-to-knee test in one, and abnormal tandem gait in one, indicating the presence of subclinical dysfunction. Therefore, we further explored the NfL levels in preSCA2. We calculated the time to the estimated age of ataxia onset based on expanded CAG repeat length and age at the time of evaluation, as previously established. Levels of serum NfL in preSCA2 were higher close to the predicted age of ataxia onset, as revealed by a linear regression between level of NfL and time from expected age of onset ($R^2 = 0.59, p = 0.01$; Figure 5A). To compare preSCA2 with controls at the same age, measured NfL levels were expressed as NfL z-score, which were calculated as the difference
between the measured NFL level and the NFL level estimated for controls at the same age and then were standardized relative to the NFL distribution in controls (Figure 5B). The NFL z-score were higher close to expected age of onset in preSCA2 ($R^2 = 0.43$, $p = 0.04$; Figure 5C), not overlapping the 95% confidence interval of controls (z-score > 1.96) already 4.8 years before the estimated age of onset. This meant the NFL levels in preSCA2 were elevated significantly 4.8 years before ataxia onset compared with controls. Residual plots for the linear regression of serum NFL and its z-score exhibited a random scatter (Figure 5D).

Discussion

This study identified that serum NFL could be a great alternative of CSF NFL in SCA2, although NFL is CNS-origin. Serum NFL was significantly higher not only at the manifest, but also at the preclinical stage of SCA2 compared to the controls. Moreover, NFL level was higher with preclinical individuals approaching to the onset of ataxia. The levels of serum NFL were correlated with disease severity, in terms of both ataxia symptoms and non-ataxia signs. These findings suggest that serum NFL is a potential biomarker of disease onset and severity in SCA2.

Neurofilaments are particularly abundant in axons with constant low-level release from axons in an age-dependent manner, and are essential for structural stability and radial growth of axons, thereby achieving an optimal nerve conduction velocity. NFL is the most abundant and soluble neurofilament subunit, hence the most reliably measurable one in biofluids. The abnormal elevated levels in either CSF or serum are acknowledged as markers of axonal injury, axonal loss, and neuronal death. With regard to the neuropathology of SCA2, the abnormally elongated polyglutamine tract encoded by a CAG expansion in the ATXN2 gene is believed to drive pathogenesis. Whereas the role of accumulation and aggregation of polyglutamine proteins is yet to be fully understood. Pathological changes usually occur in cerebellum, brainstem,
basal ganglia, and peripheral nerves, eventually leading to neuronal dysfunction, loss and death throughout the affected brain regions in patients with SCA2. Therefore, the loss of cerebellum volumes underlying the neuropathological changes in SCA2 were associated with the elevation of NfL levels resulting from axonal loss. The released NfL level in CSF was 60-fold higher than that in serum in this study, roughly in accordance with the previous report. As a consequence, NfL changes in CSF might be more responsive and sensitive in identifying a neurodegenerative progression. Blood NfL measurement, however, would be more feasible and ideal due to its easier accessibility and lower invasiveness. Furthermore, our results demonstrated that the discriminatory power of NfL measurement in serum was slightly inferior to that in CSF but sufficiently excellent, laying a theoretical foundation for the application of serum.

In this study, the elevation of NfL level was found already in the preclinical stage of the disease, and preceded the projected conversion to manifest stage by 4.8 years in SCA2. Those might be consistent with the presence of subclinical dysfunction and degeneration of the cerebellum and other neural structures before the onset of ataxia, of which brainstem, basal ganglia and peripheral nerves are notable, indicating NfL measurement as a biomarker to predict the time to onset in preclinical individuals. In addition, in a longitudinal study on SCA2 individuals at risk, the occurrences of cramps and sensory symptoms were most frequent respectively in 5 years and 4 years before ataxia onset; mean onset time between cramps and ataxia was 6.5 years; mean time from sensory symptoms onset to ataxia onset was 4.27 years, which were close to 4.8 years in this study. In other neurodegenerative diseases like HD and SCA3, NfL was also found to be elevated in presymptomatic individuals. In longitudinal studies on familial Alzheimer’s disease (AD), NfL could discriminate preclinical individuals from controls as early as one or two decades before estimated
Those studies suggested NfL measurement had the potential ability to capture the onset of axonal degeneration and monitor the subclinical progression in the preclinical stage, aiding to stratify preclinical individuals and figure out the optimal opportunity of therapeutic intervention. Longitudinal investigations on NfL measurement involving preclinical SCA2 with larger samples and multiple populations are in demand to confirm our results.

A recently prospective study enrolling 13 patients with SCA2 found NfL levels were higher in patients and could predict disease progression. Those findings were consistent with our results in terms of diagnostic value of NfL, and further highlighted its importance as a prognostic marker. That work had definite advantages for its two-year prospective analysis and relatively broad enrollment of several SCA genotypes. Our study had unique advantages as follows: (1) As 59 SCA2 individuals and 92 controls were enrolled, our study provided a more powerful evidence. (2) We included a broader range of SCA2 individuals including preclinical individuals, and found NfL was higher close to the estimated age of onset in preclinical SCA2. (3) As NfL is CNS-origin, we analyzed NfL levels in CSF, and identified that serum NfL could be a great alternative of CSF NfL in SCA2. Consequently, our findings extend the knowledge of NfL in SCA2, and provide new and independent insights into the role of NfL as a potential biomarker of capturing onset of ataxia and assessing disease severity.

Since the diagnostic value of elevated NfL level has been found in a variety of neurodegenerative diseases (summarized in eTable 4), NfL is a sensitive but unspecific biomarker of axonal damage. However, NfL seems to function in the differential diagnosis between Parkinson’s disease (PD) and atypical parkinsonian disorders. Also, the elevation of NfL level was reported to be a discriminative biomarker between frontotemporal dementia (FTD) and primary psychiatric disorders.
As a result, the potential diagnostic value of NfL does not consist in the ability to distinguish between neurological diseases with similar degree of axonal loss, but rather with a different degree of axon loss and disease severity. In addition to diagnostic value, several longitudinal studies have revealed that NfL might have important prognostic implications. In HD and PD, baseline level of NfL could predict clinical progression\textsuperscript{16, 39}. Furthermore, the value of NfL as a dynamic marker of clinical progression was highlighted in a series of neurodegenerative diseases including FTD, AD, and PD\textsuperscript{40-42}. That was not supported, however, in ALS and SCAs, as NfL maintained a relatively constant expression during follow-up\textsuperscript{36, 43}. The controversy might be relevant to different affected tissues releasing NfL underlying different neuropathology, and involvement of clearing mechanisms\textsuperscript{44}. Moreover, the dynamics of NfL might be more sensitive to early stage of neurodegeneration and more suitable to capture disease conversion, as indicated by longitudinal studies of AD and genetic FTD \textsuperscript{34, 40}. Despite of the limitation of non-specificity, NfL may still be utilized in many potential contexts, such as a tool as an outcome measure, tracking disease progression and treatment response. Therefore, studies on the association between longitudinal changes in NfL and clinical assessments, especially radiological measures, are encouraged in SCA2.

As a quantitative measure of ongoing axonal injury, NfL level is unable to help locate specific site of damage. On one hand, the lack of anatomical characterization of NfL suggests that its measurement in CSF and blood cannot replace MRI in the disease evaluation. On the other hand, NfL measurement indeed may provide more indications on the degree of ongoing axonal damage in normal-appearing MRI, especially in preclinical individuals. Therefore, NfL measurement cannot be used alone as a substitute for MRI assessment, but could be used as a complementary tool for detecting and monitoring axonal damage.
In summary, our results revealed that NfL levels were elevated in individuals with SCA2 in combined biofluids including CSF and serum. In preclinical individuals, NfL levels were higher close to the estimated age of onset. In individuals with SCA2, NfL levels were correlated with disease intensity, reflected by rating scales and neuroimaging changes. Therefore, we identified NfL measurement as a potential biomarker in SCA2. The value of NfL levels might lie in their potential to capture the onset of ataxia, monitor disease severity and progression, and further aid in stratification and therapeutic response for upcoming treatment trials. Nevertheless, our study had several limitations. First, the sample size was relatively small because of this rare disease. Second, longitudinal assessment of NfL levels, especially involving more preclinical individuals, is warranted to confirm our cross-sectional findings. Third, MRI examinations of preclinical individuals were lacking due to the participants’ personal willingness. In addition, considering that NfL levels are influenced by age, it is crucial to establish an age-dependent reference range in a large and multicenter cohort of healthy individuals before the application of NfL.

Appendix 1: Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lu Yang,</td>
<td>Zhejiang University,</td>
<td>Acquired, analyzed and interpreted the data; drafted the manuscript for intellectual content</td>
</tr>
<tr>
<td>MD</td>
<td>Hanghou, China</td>
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</tr>
<tr>
<td>Ya-Ru Shao,</td>
<td>Zhejiang University,</td>
<td>Acquired, analyzed and interpreted the data</td>
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<tr>
<td>MD</td>
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<td>Xiao-Yan Li,</td>
<td>Zhejiang University,</td>
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<tr>
<td>Yin Ma,</td>
<td>Zhejiang University,</td>
<td>Administrative, technical, and material</td>
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<td>Hanghou, China</td>
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<td>Name</td>
<td>Institution</td>
<td>Contribution</td>
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<td>Yi Dong, MD, PhD</td>
<td>Zhejiang University, Hanghou</td>
<td>Acquired, analyzed and interpreted the data</td>
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<td>Zhi-Ying Wu, MD, PhD</td>
<td>Zhejiang University, Hanghou</td>
<td>Designed and conceptualized study; acquired, analyzed and interpreted the data; critical revision of the manuscript for important intellectual content</td>
</tr>
</tbody>
</table>

References


42. Mollenhauer B, Dakna M, Kruse N, et al. Validation of Serum Neurofilament Light Chain as a
**Table 1** Demographic, clinical characteristics and NfL levels of participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group A</th>
<th>Group B</th>
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<td></td>
<td>Control</td>
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<tr>
<td>n</td>
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<td>23</td>
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<tr>
<td>Age, years</td>
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<td>Female, n (%)</td>
<td>10(50%)</td>
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<td>Expanded CAG repeat</td>
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<td>Serum NfL, pg/mL</td>
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<td>2187.86(1396.90-610.67)</td>
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<td>Brainstem volume, %</td>
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</tbody>
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ICARS, International Cooperative Ataxia Rating Scale; INAS, Inventory of Non-Ataxia Signs; N/A, not applicable; NfL, neurofilament light; PreSCA2, preclinical individuals with SCA2; SARA, Scale for the Assessment and Rating of Ataxia. Values are described as mean (standard deviation) if
normally distributed, or median (interquartile range) if non-normally distributed. Expanded and normal CAG repeat length are specially described as median (range). The cerebellum and brainstem volumes are expressed as percentages of total intracranial volumes.

**Figure 1. Assessments for potential confounding variables for neurofilament light (NfL).** (A, C) Correlations between age and (A) CSF NfL and (C) serum NfL. (B, D) Correlations between expanded CAG repeat length and (B) CSF NfL and (D) serum NfL. Correlations are represented by $r$ and $p$ values generated from Spearman rank correlation. $r$ and $p$ values were generated using either controls only (grey) or individuals with SCA2 (colored) only. Only individuals with SCA2 were included in the association between expanded CAG repeat length and NfL as controls do not carry expanded CAG repeat.
Figure 2. Neurofilament light (NfL) levels of manifest SCA2 and controls in Group A, and ROC curves for discrimination. (A, C) NfL levels in CSF and matched serum were significantly higher in manifest SCA2 than in controls. The NfL levels are shown by boxplots with Tukey whiskers. (B, D) Residual NfL levels in CSF and matched serum were significantly higher in manifest SCA2 than in controls. Residuals were generated from the regression of CSF NfL or serum NfL on age representing the component of CSF NfL or serum NfL not explained by age. The residual NfL levels are shown by boxplots with Tukey whiskers. (E) A very high degree of consistency was identified between CSF and serum NfL levels. (F) The area under a ROC curve (AUC) gives a measure of a test’s discriminatory ability, where 0.8 indicates an 80% probability of the test giving correct answer, and 1 indicates a 100% probability. CSF NfL had essentially perfect accuracy to distinguish between manifest SCA2 and controls. And serum NfL exhibited excellent accuracy. NfL values were natural log-transformed for statistical analyses and are displayed geometrically.
Figure 3. Serum neurofilament light (NfL) levels of controls and individuals with SCA2 in Group B, and ROC curves for discrimination. (A, B) Serum NfL and residual serum NfL were higher in manifest SCA2 than in controls and preclinical individuals with SCA2 (preSCA2), and also higher in preSCA2 than in controls. Residuals were generated from the regression of serum NfL on age representing the component of serum NfL not explained by age. The NfL levels and residual NfL levels are shown by boxplots with Tukey whiskers. NfL values were natural log-transformed for statistical analyses and are displayed geometrically. *p values were generated from multiple linear regressions, surviving Bonferroni correction. (C) Serum NfL had high accuracy to distinguish among manifest SCA2, preSCA2 and controls, and between SCA2 individuals and controls.
**Figure 4. Association between serum neurofilament light (NfL) levels and disease severity in individuals with SCA2.** Elevated Serum NfL levels were significantly associated with worse SARA scores (A), worse ICARS scores (B), worse INAS scores (C), and smaller cerebellum volumes (D). The manifest SCA2 were classified into two subgroups based on median SARA scores and clinical features, including Stage 1 (3 ≤ SARA < 11) and Stage 2 (SARA ≥ 11) patients. All volumetric measures were calculated as percentages of total intracranial volumes. Partial $p$ and $r$ values were obtained after adjustment for age.
Figure 5. Association between serum neurofilament light (NfL) and time from expected onset in preclinical SCA2 (preSCA2). (A) Serum NfL levels were significantly higher close to the predicted age of ataxia onset in preSCA2, as revealed by a linear regression between NfL levels and time from expected onset. (B) To compare preSCA2 with controls at the same age, measured NfL levels were expressed as NfL z-scores. Difference between the measured NfL level (green dot) and the NfL level estimated for controls at the same age (solid gray line) is visualized by the length of vertical line. Standardization of this difference relative to the NfL distribution in controls yields the individual NfL z-score. (C) The NfL z-score was significantly higher close to the expected age of onset in preSCA2, not overlapping the 95% confidence interval of controls (i.e. z-score > 1.96, dashed black line) already 4.8 years respectively before the estimated age of onset. (D) Residual plot for the linear regression of serum NfL z-score exhibits a random scatter.
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