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ORIGINAL ARTICLE

Serum neurofilament light chain in distinct phenotypes of amyotrophic lateral sclerosis: A longitudinal, multicenter study

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Abstract

Objective: To assess the performance of serum neurofilament light chain (sNfL) in clinical phenotypes of amyotrophic lateral sclerosis (ALS).

Methods: In 2949 ALS patients at 16 ALS centers in Germany and Austria, clinical characteristics and sNfL were assessed. Phenotypes were differentiated for two anatomical determinants: (1) upper and/or lower motor involvement (typical, typMN; upper/lower motor neuron predominant, UMNp/LMNp; primary lateral sclerosis, PLS) and (2) region of onset and propagation of motor neuron dysfunction (bulbar, limb, flail-arm, flail-leg, thoracic onset). Phenotypes were correlated to sNfL, progression, and survival.

Results: Mean sNfL was - compared to typMN (75.7 pg/mL, $n = 1791$) - significantly lower in LMNp (45.1 pg/mL, $n = 413$), UMNp (58.7 pg/mL $n = 206$), and PLS (37.6 pg/mL, $n = 84$). Also, sNfL significantly differed in the bulbar (92.7 pg/mL, $n = 669$), limb (64.1 pg/mL, $n = 1305$), flail-arm (46.4 pg/mL, $n = 283$), flail-leg (53.6 pg/mL, $n = 141$), and thoracic (74.5 pg/mL, $n = 96$) phenotypes. Binary logistic regression analysis showed highest contribution to sNfL elevation for faster progression (odds ratio [OR] 3.24) and for the bulbar onset phenotype (OR 1.94). In contrast, PLS (OR 0.20), LMNp (OR 0.45), and thoracic onset (OR 0.43) showed reduced contributions to sNfL. Longitudinal sNfL (median 12 months, $n = 2862$) showed minor monthly changes (<0.2%) across all phenotypes. Correlation of sNfL with survival was confirmed ($p < 0.001$).

Thomas Meyer and Marie Dreger contributed equally to this work.

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Conclusions: This study underscored the correlation of ALS phenotypes – differentiated for motor neuron involvement and region of onset/propagation – with sNfL, progression, and survival. These phenotypes demonstrated a significant effect on sNfL and should be recognized as independent confounders of sNfL analyses in ALS trials and clinical practice.

KEYWORDS
amyotrophic lateral sclerosis, biomarker, NfL, phenotype, serum neurofilament light chain

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal degenerative disorder of motor neurons [1, 2]. Clinical symptoms and individual prognosis are highly variable and related to distinct phenotypes [3–8]. In recent years, neurofilament light chain (NfL) has emerged as a prognostic biomarker in patient management and clinical research [9–15]. Specifically, NfL concentrations in cerebrospinal fluid (CSF) and serum (sNfL) are robust indicators of axonal damage in ALS. NfL levels significantly correlate with disease progression as measured by the ALS Functional Rating Scale-Revised (ALSFRS-R) and, most importantly, with survival [16, 17]. Furthermore, NfL has been introduced as an endpoint in clinical trials as an early indicator of treatment response [18–20].

Despite the established role of NfL as a prognostic marker, an area of uncertainty concerns the impact of distinct clinical phenotypes on NfL. Reportedly, dominant involvement of upper or lower motor neurons may modify NfL levels [21]. In this context, NfL levels in the phenotypes of typical (mixed) motor neuron degeneration in contrast to predominant motor neuron involvement (e.g., upper/lower motor neuron predominant variants of ALS) are of interest. Also, the site of onset and propagation pattern might be associated with different extents of neuroaxonal damage and sNfL elevation. As such, NfL in phenotypes with typical limb or bulbar onset may differ from phenotypic variants with distinct regional onset (e.g., thoracic onset) or protracted propagation of motor neuron dysfunction (e.g., flail-arm and flail-leg phenotypes) [22–24].

This issue is of relevance for the design of clinical trials when using sNfL as stratification criterion for randomization. Inclusion of NfL in multivariate trial models is thought to control for the rate of disease progression, allowing an earlier detection of biomarker response [20, 25–27]. However, such a strategy may be challenged by the hypothesis that phenotypes are in fact covariates of sNfL, as this would require to also control for clinical phenotypes. Another research question refers to the performance of NfL in the temporal course of ALS. A few longitudinal studies offer inconsistent results of either stable or moderately increasing NfL concentrations during disease progression [28, 29]. Clarification of these questions is obligatory for the refinement of NfL as a prognostic marker in clinical practice and trials, including its implementation as a biomarker for treatment response, drug safety, and phenoconversion [20, 25, 30].

To evaluate the contribution of sNfL to existing models of disease progression, a multicenter prospective study was performed. The aims of the present study were to (i) extend the sNfL data repository in terms of number of participants and follow-up measurements, (ii) to assess ALS phenotypes in the studied cohort, (iii) to correlate the phenotypes with sNfL, progression, and survival, and (iv) to analyze the effect size to which phenotypes contribute to sNfL levels.

METHODS

Study design

This observational study was conducted as a prospective, multicenter, longitudinal cohort study. The investigation was reported according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) criteria [31].

Participants and definition of cohorts

Participants met the diagnostic criteria of ALS with reference to the Gold Coast criteria [32]. Phenotypic classification as described below was made by experienced neurologists at the participating study centers. A definition of studied cohorts and subgroups is provided in Figure 1.

Setting

Recruitment

Following informed consent, patients were recruited at 16 multidisciplinary ALS centers in Germany and Austria between April 2019 and September 2023.

Data collection

Blood samples for sNfL analysis were obtained in time intervals of 5 to 7 months. Classification of the phenotype and rating of ALSFRS-R was performed by a qualified evaluator. Additional ALSFRS-R-SE

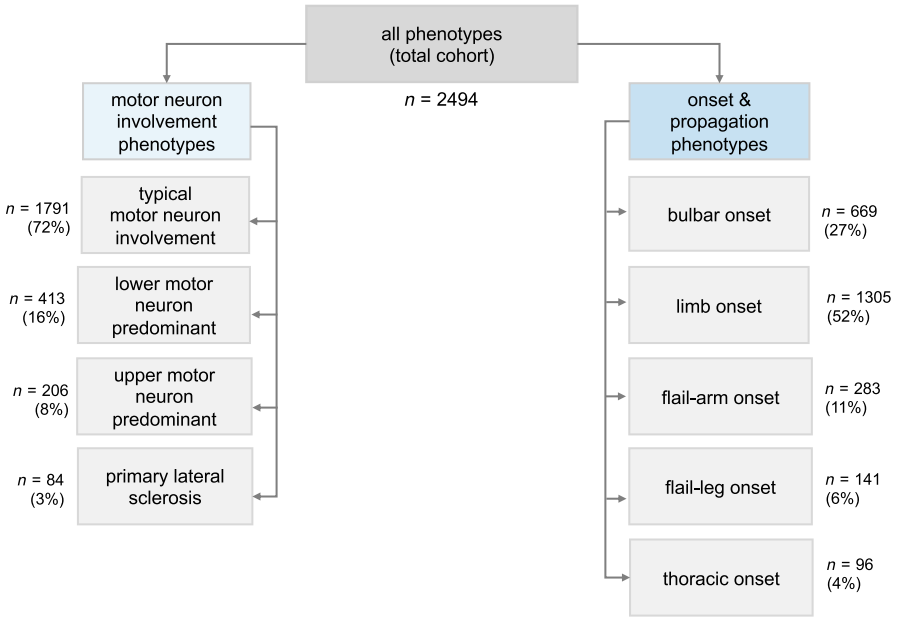

FIGURE 1 Studied amyotrophic lateral sclerosis cohort and patient stratification. For phenotyping, two anatomic determinants were distinguished: the variable dysfunction of upper and lower motor neurons (motor neuron phenotypes) and onset and propagation of motor neuron dysfunction throughout the body regions (onset and propagation phenotypes).

data were assessed by self-rating using a mobile application ("ALS-App") [33, 34]. At the end of study, an update of phenotypes and survival was performed.

Biosample collection

Blood samples were collected, centrifuged, aliquoted, and shipped to the ALS center in Berlin (Germany) where the core facility for NfL analysis was located.

sNfL analysis

Measurement of sNfL concentration was done using single molecule array (SIMOA) technology (HD-X Analyzer; Quanterix Inc., Billerica, MA, USA) using the commercially available NfL advantage kit.

Protocol approvals and registrations

The study protocol was ethically approved under numbers EA2/168/20 and EA1/219/15. A signed informed consent form was obtained from all study participants.

Variables

Demographic and clinical characteristics

The following demographic and clinical characteristics were collected: age at disease onset, sex, disease duration (number of months between disease onset and beginning of observation period), and survival (number of months between disease onset and death) (Tables 1 and 2).

ALS phenotypes

Phenotypes were classified according to their two anatomical determinants as previously described. [35–42]. As such, two domains of phenotypes were distinguished:

(A) Motor neuron involvement phenotypes reflecting the variable dysfunction of upper and lower motor neurons [35].

(i) Typical motor neuron involvement (typMN): balanced (mixed) upper motor neuron (UMN) and lower motor neuron (LMN) dysfunction.
(ii) Lower motor neuron predominant phenotype (LMNp): predominant LMN dysfunction whereas discrete UMN dysfunction is also present.
(iii) Upper motor neuron predominant phenotype (UMNp): predominant UMN dysfunction whereas discrete LMN dysfunction is also present.
(iv) Primary lateral sclerosis (PLS): pure upper motor neuron (UMN) dysfunction without lower motor neuron (LMN) involvement.

(B) Onset and propagation phenotypes reflecting the region of onset and the propagation of motor neuron dysfunction throughout the body regions [35].

(i) Bulbar onset: onset in the bulbar region, followed by typical propagation to the cervical, thoracic, and lumbar regions.
(ii) Limb onset: onset of motor neuron dysfunction in a limb region, followed by typical propagation to the bulbar, cervical, thoracic, and lumbosacral regions.

TABLE 1 Motor neuron involvement phenotypes – clinical characteristics and neurofilament light chain.

Parameter	Total	Typical	LMNp	UMNp	PLS	
Patients	2949	1791 (72%)	413 (17%)	206 (8%)	84 (3%)	*P*-value
Demographics						
Age (years)	64	64	66	62	62	0.065
	(57–72)	(57–71)	(58–73)	(56–72)	(56–71)	
Male/female	1451 (58%)	986 (55%)	307 (74%)	114 (55.3%)	44 (52%)	<0.001
	1043 (42%)	805 (45%)	106 (26%)	92 (44.7%)	40 (48%)	
Clinical characteristics						
Duration (months)	18	16	27	27	57	<0.001
	(10–39)	(10–31)	(13–59)	(12–72)	(26–93)	
ALSFRS-R	36	37	35	34	37	<0.001
	(29–41)	(30–42)	(27–41)	(26–40)	(31–42)	
ALS-PR	0.53	0.58	0.42	0.44	0.21	<0.001
	(0.28–1)	(0.33–1)	(0.22–0.77)	(0.24–0.94)	(0.12–0.39)	
Survival						
Deceased	573 (23%)	460 (26%)	82 (20%)	28 (14%)	3 (4%)	<0.001
Survival (months)	36	33	44	48	86	<0.001
	(23–61)	(22–52)	(26–83)	(28–90)	(43–122)	
Neurofilament light chain (NfL)						
sNfL (pg/mL)	67.89	75.68	45.05	58.73	37.66	<0.001
	(39–114)	(48–123)	(26–81)	(32–116)	(20–63)	
sNfL *Z*-score	3.09	3.19	2.75	3.04	2.62	<0.001
	(2.58–3.43)	(2.79–3.43)	(1.96–3.16)	(2.36–3.43)	(1.78–3.04)	

Note: Categorical variables are given as number and percentage. Continuous variables are given as median (25th–75th percentile). NfL *Z*-score, age-adjusted sNfL *Z*-scores in reference to open-access database of healthy controls.

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS Functional Rating Scale-Revised; ALS-PR, ALS progression rate; LMNp, lower motor neuron predominant phenotype; PLS, primary lateral sclerosis; sNfL, serum neurofilament light chain; UMNp, upper motor neuron predominant phenotype.

(iii) Flail-arm onset: onset in the upper limbs, followed by protracted propagation to the bulbar, thoracic, and lumbar regions.
(iv) Flail-leg onset: onset of motor neuron dysfunction in the lower limbs, followed by protracted propagation to the thoracic, cervical, and bulbar region.
(v) Thoracic onset: onset in the thoracic region (with respiratory symptoms and/or trunk instability), followed by propagation to the bulbar, cervical, and lumbosacral regions.

ALS Functional Rating Scale-Revised (ALSFRS-R)

The ALSFRS-R is a 12-item disease-specific instrument that measures functional impairment in ALS (Supplement – Methods) [43].

ALS progression rate (ALS-PR)

ALS-PR was measured by the monthly change in the ALSFRS-R sum score and calculated using the following equation: 48-ALSFRS-R divided by disease duration (months) [44].

Neurofilament light chain in serum (sNfL)

The measurement of sNfL concentration was in picograms per milliliter (pg/mL). To investigate longitudinal performance of sNfL, the difference in sNfL concentration was calculated, and divided by the number of months from the baseline to the follow-up measurement.

Statistical methods

Statistical analyses were performed using SPSS (SPSS Statistics for Windows, Version 27.0; IBM Corp., Armonk, NY, USA). GraphPad Prism (Version 9.0.0 for Windows; GraphPad Software, San Diego, CA, USA) was used for graphical representation of data. Continuous variables were assessed for normality using the Shapiro–Wilk test and described accordingly as mean ± standard deviation (SD) or median and interquartile range (IQR) (25th–75th percentile). Categorical variables are provided as absolute numbers (*n*) and percentages (%). Statistical significance is defined as $p < 0.05$.

TABLE 2 Onset and propagation phenotypes – clinical characteristics and neurofilament light chain.

Parameter	All onsets 2949	Limb onset 1305 (52%)	Bulbar onset 669 (27%)	Flail-arm onset 283 (11%)	Flail-leg onset 141 (6%)	Thoracic onset 96 (4%)	
Demographics							
Age (years)	64	62	66	63	62	69	<0.001
	(57–72)	(54–70)	(60–74)	(57–71)	(56–70)	(62–74)	
Male/female	1451 (58%)	789 (61%)	284 (42%)	221 (78%)	82 (58%)	75 (78%)	<0.001
	1043 (42%)	516 (39%)	385 (58%)	62 (22%)	59 (42%)	21 (22%)	
Clinical characteristics							
Duration (months)	18	20	14	19	29	15	<0.001
	(10–39)	(11–45)	(9–25)	(10–49)	(17–53)	(9–30)	
ALSFRS-R	36	36	37	37	38	32	<0.001
	(29–41)	(29–41)	(30–42)	(29–43)	(31–42)	(25–38)	
ALS-PR	0.53	0.5	0.63	0.43	0.34	0.9	<0.001
	(0.28–1.0)	(0.27–0.95)	(0.36–1.1)	(0.23–0.83)	(0.18–0.51)	(0.56–1.62)	
Survival							
Deceased	573 (23%)	270 (21%)	197 (29%)	52 (18%)	26 (18%)	28 (29%)	<0.001
Survival (months)	36	39	30	38	48	30	<0.001
	(23–61)	(24–66)	(20–46)	(25–69)	(35–77)	(19–43)	
Neurofilament light chain (NfL)							
sNfL (pg/mL)	67.89	64.09	92.74	46.4	53.6	74.75	<0.001
	(39–114)	(37–108)	(58–152)	(29–78)	(31–84)	(48–103)	
sNfL *Z*-score	3.09	3.09	3.24	2.75	2.95	3.02	<0.001
	(2.58–3.43)	(2.58–3.43)	(2.93–3.54)	(2.1–3.24)	(2.51–3.24)	(2.75–3.24)	

Note: Categorical variables are given as number and percentage. Continuous variables are given as median (25th–75th percentile). NfL *Z*-score, age-adjusted sNfL *Z*-scores in reference to open-access database of healthy controls.

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS Functional Rating Scale-Revised; ALS-PR, ALS progression rate; LMNp, lower motor neuron predominant phenotype; PLS, primary lateral sclerosis; sNfL, serum neurofilament light chain; UMNp, upper motor neuron predominant phenotype.

An age-adjusted sNfL *Z*-score was calculated using a reference database of a healthy control population as described previously [17, 45]. Differences in age, disease duration, ALSFRS-R, ALS-PR, sNfL concentration, and sNfL *Z*-scores between phenotypes were analyzed using the Kruskal–Wallis test. To analyze differences between two categorical variables the Chi-square test was used. Binary logistic regression was performed to determine the concurrent effect of age, phenotype, and ALS-PR on sNfL concentrations. sNfL served as an independent variable for the logistic regression analysis. For grouping of sNfL, the cohort was first split into three equally sized groups with low, intermediate, and high sNfL concentrations (cut-off values of sNfL at 49.3 and 93.5 pg/mL). The group of high sNfL was then compared with the groups of intermediate and low sNfL combined. Age, ALS-PR, and phenotype were introduced as covariates in the logistic regression analysis. For phenotype analysis the typMN and limb onset phenotypes served as reference. Log-rank tests were performed to calculate survival differences between sNfL subgroups and different phenotypes. A multivariate Cox proportional hazard regression analysis was performed to assess the contribution of age, ALS-PR, sNfL, and phenotype to survival.

RESULTS

Clinical characteristics and distribution of phenotypes

A total of 2949 ALS patients were included in the study (Figure 1). Demographic and clinical characteristics are given in Tables 1 and 2. With respect to UMN and LMN involvement, most patients ($n = 1791$, 72%) showed the typMN phenotype, followed by LMNp ($n = 413$, 17%), UMNp ($n = 206$, 8%), and PLS ($n = 84$, 3%). Some 1974 subjects (77%) showed the limb or bulbar onset phenotypes whereas the remaining patients presented with flail-leg ($n = 141$, 6%), flail-arm ($n = 283$, 11%), or thoracic onset phenotypes ($n = 96$, 4%).

sNfL in phenotypes

Motor neuron involvement phenotypes were significantly correlated with sNfL ($p < 0.001$) (Table 1, Figure 2). sNfL was found to be highest in the typMN phenotype, followed by UMNp, LMNp, and PLS. Also, sNfL concentration differed significantly between distinct onset/propagation phenotypes with the highest sNfL levels in the

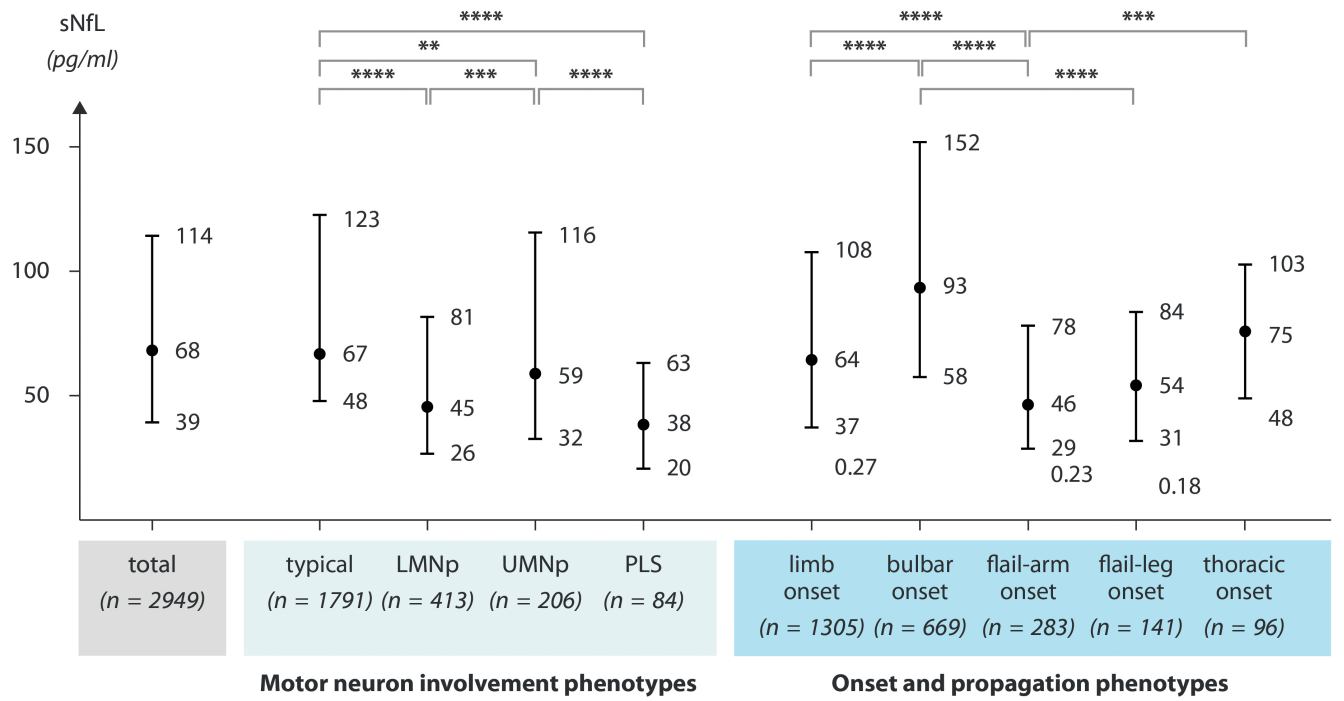

FIGURE 2 Serum neurofilament light chain in correlation to phenotypes. For phenotyping, two anatomical determinants of motor neuron dysfunction were distinguished: (1) motor neuron involvement phenotypes with variable involvement of upper and lower motor neuron dysfunction and (2) onset and propagation phenotypes with distinct onset and propagation of motor neuron dysfunction throughout the body regions. LMNp, lower motor neuron predominant phenotype; PLS, primary lateral sclerosis; sNfL, serum neurofilament light chain; UMNp, upper motor neuron predominant phenotype. The bar indicates the median, hinges extend from the 25th to the 75th percentile. Significance levels are indicated as: $^{**}p \leq 0.05$, $^{***}p \leq 0.01$, $^{****}p \leq 0.001$; non-significant differences are not shown.

bulbar onset and thoracic phenotypes, followed by limb onset, flail-arm and flail-leg onset phenotypes (Table 2, Figure 2, Figure S1).

ALS progression rate in phenotypes

ALS-PR differed significantly between motor neuron phenotypes ($p < 0.001$). Faster progression (ALS-PR 0.58) was observed in the typMN phenotype, followed by the UMNp (0.44), LMNp (0.42), and PLS (0.21) phenotypes (Table 1, Figure 3). Also, onset/propagation phenotypes showed significant differences in ALS-PR with faster progression in thoracic onset ALS (ALS-PR 0.9) followed by bulbar onset (ALS-PR 0.63). Conversely, flail-leg and flail-arm phenotypes demonstrated slower progressing ALS (ALS-PR 0.34 and 0.43, respectively; Table 2, Figure 3, Figure S2, Supplement – Results).

Survival in phenotypes

Survival of patients with distinct ALS phenotypes of motor neuron involvement differed significantly ($p < 0.0001$) (Table 1, Figure 4). Thus, significant differences were found in the survival distributions between typMN versus. PLS, UMNp, and LMNp. Also, the onset/propagation phenotypes revealed survival differences such as between limb versus bulbar onset, limb versus thoracic onset, and bulbar versus flail-leg and flail-arm onset phenotypes (Table 2, Figure 4, Supplement – Results).

Contribution of phenotypes to sNfL elevation

ALS-PR showed the highest contribution to sNfL elevation (OR 3.252, $p < 0.001$) (Figure 5). Moreover, distinct phenotypes were found to be additional covariates of sNfL increase. When using the typMN phenotype as reference, PLS (OR0.208, $p < 0.001$) and the LMNp phenotype (OR 0.456, $p < 0.001$) revealed a lower contribution to sNfL elevation (Figure 5 and Table S1). Also, in the onset and propagation phenotypes, a different impact of distinct phenotypes on sNfL elevation was found. When referencing the limb onset phenotype, the bulbar onset phenotype showed a higher contribution to sNfL elevation (OR 1.942, $p < 0.001$). Conversely, the flail-arm (OR 0.495, $p < 0.001$) and thoracic onset (OR 0.436, $p < 0.003$) phenotypes revealed a reduced contribution to sNfL elevation (Figure 5 and Table S1).

Temporal course of sNfL in total cohort and distinct phenotypes

In the total cohort, 2862 follow-up sNfL measurements were available (Table 3). When comparing the last available sNfL value with the baseline measurement (median duration 12 months), sNfL

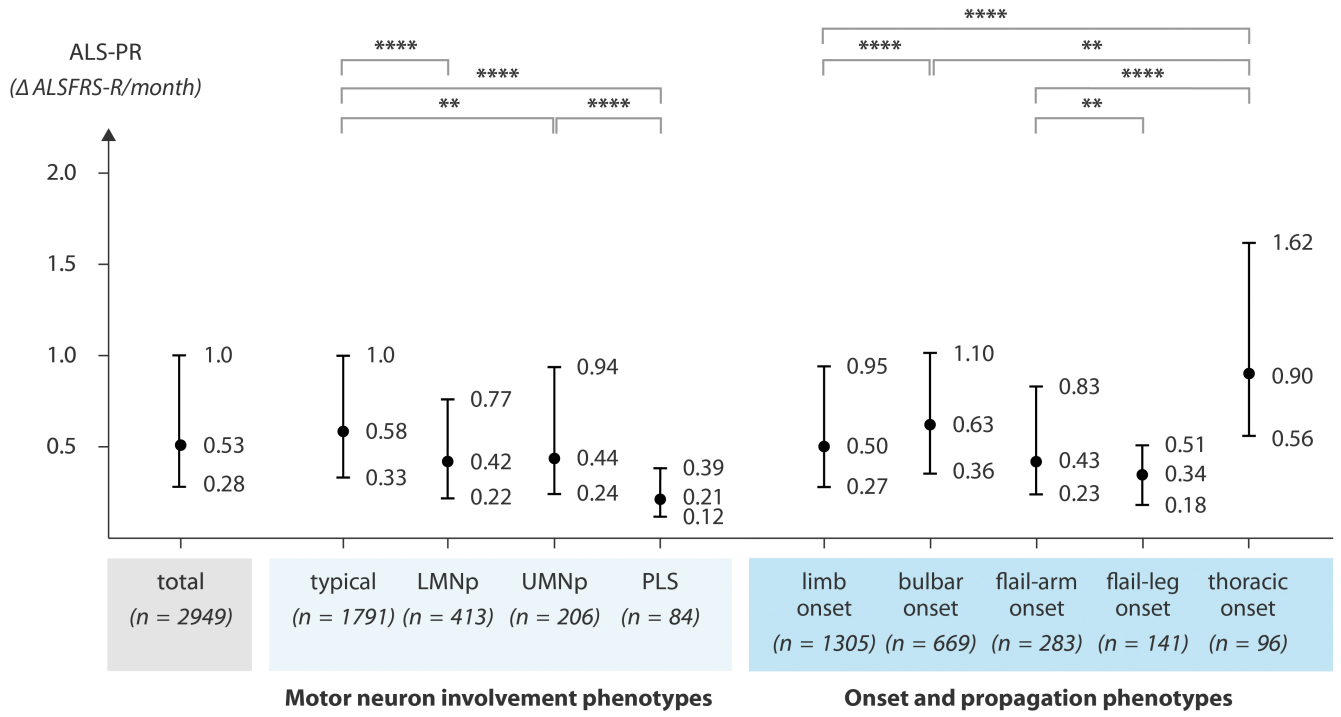

FIGURE 3 Amyotrophic lateral sclerosis progression rate in correlation to phenotypes. For phenotyping, two anatomical determinants of motor neuron dysfunction were distinguished: (1) motor neuron phenotypes with variable involvement of upper and lower motor neurons and (2) onset and propagation phenotypes with distinct onset and propagation of motor neuron dysfunction throughout the body regions. ALS, amyotrophic lateral sclerosis; ALS-PR, ALS progression rate; LMNp, lower motor neuron predominant phenotype; MN, motor neuron, UMNp, upper motor neuron predominant phenotype; PLS, primary lateral sclerosis; typical, upper and lower motor neuron involvement. The bar indicates the median, hinges extend from the 25th to the 75th percentile. Significance levels are indicated as: $^{**}p \leq 0.05$, $^{****}p \leq 0.0001$.

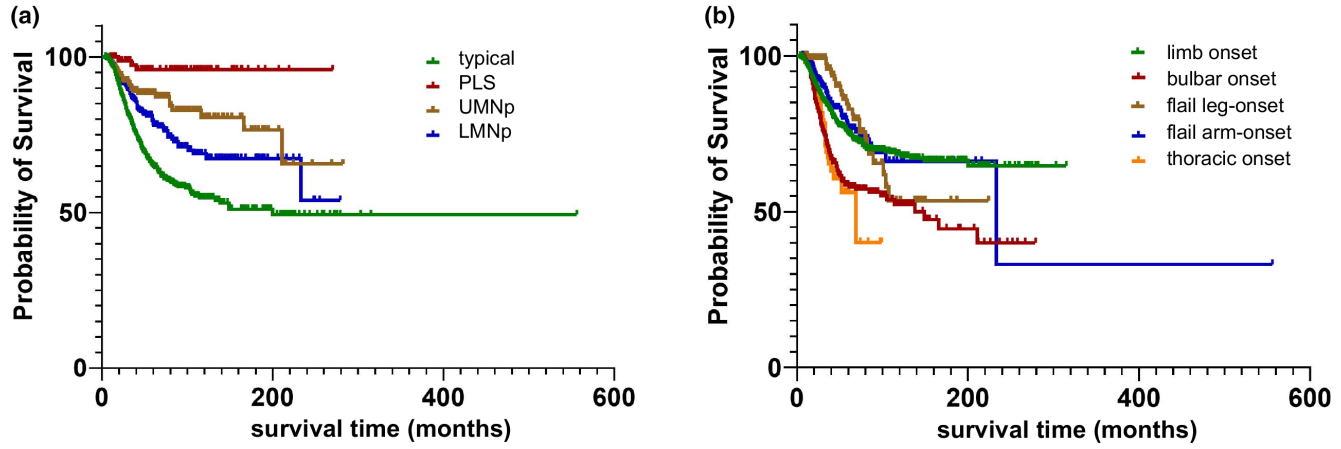

FIGURE 4 Correlation of phenotypes with survival probability. For phenotyping, two anatomical determinants of motor neuron dysfunction were distinguished: (a) motor neuron phenotypes with variable involvement of upper and lower motor neurons and (b) onset and propagation phenotypes with distinct onset and propagation of motor neuron dysfunction throughout the body regions. LMNp, lower motor neuron predominant phenotype; PLS, primary lateral sclerosis; UMNp, upper motor neuron predominant phenotype.

demonstrated stability in the temporal course (median increase 0.09 pg/mL per month; relative change 0.17%). Stratification of patients by phenotypes showed the highest sNfL change (0.69 pg/mL per month) to be in the bulbar onset phenotype (relative monthly change 0.7%). In all other phenotypes, the sNfL change was even lower (Table 3 and Figure S3).

DISCUSSION

The heterogeneity of ALS adds complexity to the interpretation of neurofilament as a prognostic biomarker. The clinical spectrum of ALS is mainly caused by phenotypes that result from the variable involvement of upper and lower motor neurons, as well as the region

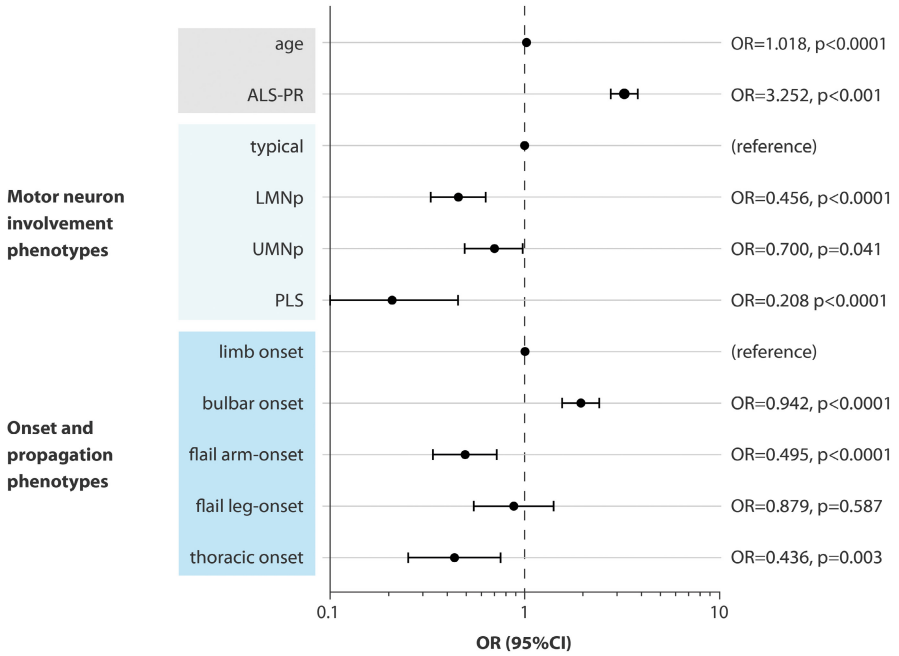

FIGURE 5 Contribution of phenotypes to elevation of neurofilament light chain (NfL). Results of binomial logistic regression analysis to determine the concurrent effect of phenotypes, age, and amyotrophic lateral sclerosis progression rate on serum neurofilament light chain (sNfL) concentrations. For motor neuron involvement phenotypes, the typical phenotype served as reference category whereas for onset/propagation phenotypes, the limb onset phenotype served as reference. Odds ratios determine the likelihood of reaching high sNfL concentrations (>93.5 pg/mL, highest third in the cohort). ALS-PR, amyotrophic lateral sclerosis progression rate; CI, confidence interval; LMNp, lower motor neuron predominant phenotype; OR, odds ratio; PLS, primary lateral sclerosis; UMNp, upper motor neuron predominant phenotype.

of onset and propagation pattern throughout the body regions [35, 36, 46]. Although the correlation between NfL and ALS progression has been demonstrated in previous studies, the effect of phenotypes on sNfL was uncertain [23]. As such, there was an open issue as to whether the considerable clinical heterogeneity expressed in different phenotypes is fully controlled by NfL [25]. To date, only a few studies have investigated the relationship between NfL and ALS phenotypes [47–49]. To our knowledge, the present work provides the most comprehensive analysis of the effect of clinical phenotypes on NfL. In line with previous reports, a strong correlation of sNfL with survival was found (Supplement – Results, Figure S4) [17].

This study revealed that sNfL concentrations, progression, and survival are correlated with distinct phenotypes. In this, our findings support and extend existing knowledge from smaller studies [22, 23, 48, 49]. Patients with typical involvement of upper and lower motor neurons showed the fastest progression, shortest survival, and highest sNfL concentrations. In contrast, the LMNp phenotype was associated with lower sNfL, slower progression, and longer survival compared with the typMN phenotype. The results were even more obvious in PLS patients that exhibited the lowest sNfL concentrations and most favorable prognosis in terms of ALS progression and survival.

When analyzing phenotypes of onset and propagation, the highest sNfL concentrations were found in bulbar and thoracic onset phenotypes (median: 92.7 and 74.7 pg/mL, respectively). Correspondingly, these phenotypes were associated with faster progression and shorter survival. Conversely, the flail-arm phenotype was associated with lower progression, reduced sNfL levels, and longer survival. Of note, the thoracic phenotype showed the fastest progression across all phenotypes but not the highest sNfL elevation. This observation contributes to the notion that NfL alone is not sufficient to predict ALS progression and must be viewed in the context of the phenotype. The contradiction between high progression and relatively low NfL is even greater when applying age correction by means of the NfL *Z*-score. The age-adaptation of sNfL (by means of the *Z*-score) came into effect as patients with the thoracic onset phenotype exhibited higher mean age (Table 2) [17, 45].

An sNfL elevation was found in both the UMNp and LMNp phenotypes. This finding supports the assumption that both upper and lower motor neuron degeneration may contribute to NfL elevation. However, the results from binomial logistic regression analysis suggest that distinct phenotypes may have different impacts on sNfL elevation. Thus, the PLS phenotype (i.e., pure motor neuron degeneration in the brain) showed the lowest effect on sNfL. Also, patients with the LMNp phenotype (i.e., predominant degeneration in the spinal cord) had lower sNfL levels compared with the typMN phenotype (i.e., degeneration in brain and spinal cord combined). Moreover, in the flail-arm and thoracic phenotypes (i.e., focal onset of motor neuron degeneration in the cervical or thoracic spinal cord) a lower contribution to sNfL elevation was found. As such, it is conceivable that sNfL elevation reflects both the dynamics of neuroaxonal lesions (ALS progression rate) and the topography

TABLE 3 Longitudinal change of serum neurofilament light chain in amyotrophic lateral sclerosis phenotypes.

Parameter	sNfL, number of measurements	sNfL, monthly change from baseline (pg/mL)	Time interval from baseline (months)	sNfL, monthly change from baseline (%)
	2862	0.09 [0.04. 0.15]	12.21 ± 8.24	0.17
Duration from baseline (months)				
1–3	155	0.21 [−1, 1.38]	2.65 ± 0.63	0.31
4–6	762	0.12 [−0.09, 0.40]	5.08 ± 0.8	0.19
7–9	421	0.29 [−0.03, 0.65]	7.9 ± 0.8	0.46
10–12	436	0.26 [0.08, 0.42]	11.06 ± 0.8	0.51
13–18	524	0.08 [−0.04, 0.24]	15.39 ± 1.74	0.17
19–24	323	0.01 [−0.06, 0.11]	21.29 ± 1.70	0.02
>24	241	0.02 [−0.03, 0.08]	31.42 ± 5.94	0.04
Motor neuron involvement phenotypes				
Typical	1870	0.26 [0.13, 0.35]	11.6 ± 8.08	0.42
LMNp	561	−0.01 [−0.1, 0.07]	13.04 ± 8.32	−0.02
UMNp	302	−0.14 [−0.31, 0]	13.34 ± 8.63	−0.32
PLS	129	0.12 [0.01, 0.29]	14.72 ± 8.32	0.43
Onset and propagation phenotypes				
Limb onset	1551	−0.01 [−0.07, 0.05]	12.71 ± 8.60	−0.02
Bulbar onset	711	0.69 [0.42, 1.14]	10.95 ± 7.66	0.95
Flail-arm onset	330	0.08 [−0.02, 0.21]	12.53 ± 7.57	0.23
Flail-leg onset	184	−0.09 [−0.36, 0.06]	13.08 ± 8.40	−0.18
Thoracic onset	86	0.15 [−0.3, 0.79]	10.51 ± 7.06	0.23

Note: Change in sNfL serum concentration (pg/mL) and relative change (%) per month from baseline.

Abbreviations: LMNp, lower motor neuron predominant phenotype; PLS, primary lateral sclerosis; sNfL, serum neurofilament light chain; UMNp, upper motor neuron predominant phenotype.

of motor neuron loss (ALS phenotypes). The results from patients with thoracic onset added another layer of complexity. Although the thoracic phenotype was associated with a lower contribution to sNfL elevation, it was associated with faster progression and shorter survival. This can be explained by the topography of motor neuron dysfunction in a critical spinal cord region that is related to hypoventilation and short survival. It is conceivable that the sNfL level alone is not only the predictor for the prognosis, but also the source region of sNfL elevation.

Concerning the interrelation of sNfL, phenotypes, and ALS progression, three aspects need to be distinguished: (i) multivariate analysis showed that distinct phenotypes show a significantly different contribution to sNfL elevation; (ii) notwithstanding the impact of phenotypes on sNfL, the strong correlation between sNfL and progression persisted within each of the phenotype cohorts; and (iii) irrespective of the clinical phenotype, the ALS-PR was the strongest determinant of sNfL concentration (OR 3.25, $p < 0.001$).

The advances of this study are two-fold. First, it further proves the significant correlation between NfL concentration and survival in a very large cohort. The reproduction of prior reports also applied to the characterization of phenotypes (with an expanded prognostic dataset). The second advancement of this study concerned the demonstration that ALS phenotypes indeed have an independent contribution to sNfL. As such, different sNfL contributions of the motor neuron involvement and onset/propagation phenotypes were found – also in phenotypes that are typically associated with each other such as the flail-leg (OR 0.45) and LMNp (OR 0.88) phenotypes. This observation supports the approach of a separate analysis of the two anatomical determinants of ALS phenotypes (motor neuron involvement vs. onset/propagation). However, there are several limitations that warrant cautiousness in the conclusions. In this study, multivariate analyses were only referenced to two phenotypes, namely the typical motor neuron involvement and the limb onset phenotype, making further reference combinations desirable. Furthermore, a more granular and longitudinal assessment of the phenotypes may be required. This necessity can arise when the initial phenotype (e.g., typical motor neuron involvement) might be blurred and replaced by another clinical presentation (e.g., LMNp) in a more progressed disease phase. Despite these limitations, the actual finding of a differential contribution of phenotypes to sNfL revealed that the biomarker is not solely driven by the different progression rates. Obviously, sNfL alone cannot resolve all the complexity resulting from clinical heterogeneity. Therefore, distinct phenotypes should still, if indeed not increasingly, be considered in prediction models and clinical trial design [50, 51].

The longitudinal stability of sNfL levels was demonstrated in a total of 2862 follow-up measurements confirming previous reports

in smaller samples [28, 29]. The total number of measurements that span more than 24 months was reasonable ($n = 241$, Table 3) but needs to be expanded in future studies. The call for more long-term data is based on a previous observation that sNfL levels decline in patients with long disease duration and invasive ventilation [17]. Although sNfL levels appear to remain stable over a longer period of time, this study also provided evidence for the greater variability of sNfL values in a short-term perspective (1–3 months) (Table 3). This phenomenon has been reported previously; however, is not well understood and needs to be further investigated [52].

The strengths of this study were the size of the cohort, the duration of data collection, the multicenter design, and the central infrastructure for sNfL analysis. Nevertheless, the study is not without limitations. Currently, there is no broader consensus on the classification or even naming of ALS phenotypes, although this issue has been addressed [53]. Therefore, this study referred to accepted phenotypic terms such as bulbar and limb onset, PLS, UMNp and LMNp, flail-arm, flail-leg, and thoracic onset [4–6, 36–42]. Only progressive muscle atrophy (PMA) was included in the LMNp phenotype but was not analyzed separately. The reason to pool both phenotypes at this point of the investigation was justified by there being too wide a scope for interpretation to classify PMA or LMNp. Future research will aim to differentiate between PMA and LMNp by the further concretization in the study protocol to assess both phenotypes. Beyond the identification of phenotypes, they were grouped according to two anatomical determinants of motor neuron dysfunction, as suggested previously [35]. As this grouping was not standard of care, a training of study sites for the classification of the phenotypes was performed. Notwithstanding the training, an inter-rater variability cannot be excluded. It was reassuring, however, that the frequency distribution of the phenotypes in this investigation was in line with previous reports, making substantial deviations unlikely [4–6, 53, 54].

In conclusion, this study underscored the correlation of distinct ALS phenotypes to progression and survival. Furthermore, clinical phenotypes pose independent variables impacting sNfL levels in ALS. These findings come with a two-sided message. The first perspective concerns the biomarker that needs to be viewed in the context of clinical phenotypes. This context is of importance for the correct interpretation of the biomarker in interventional trials and clinical practice. The second perspective refers to the principal importance of phenotypes. As sNfL is not sufficient to control for the clinical heterogeneity, the relevance of clinical phenotypes for prognostic prediction was emphasized. In future research more effort is needed to differentiate and standardize the phenotypes – in conjunction with sNfL, the most informative and robust biomarker currently available in ALS.

AUTHOR CONTRIBUTIONS

Thomas Meyer: Conceptualization; methodology; data curation; supervision; project administration; resources; validation; investigation; funding acquisition; writing – original draft; visualization. **Marie Dreger:** Conceptualization; methodology; data curation; supervision; writing – original draft; investigation; validation; visualization; software. **Torsten Grehl:** Data curation; methodology; investigation; project administration; resources; validation. **Ute Weyen:** Methodology; validation; investigation; writing – review and editing; project administration; resources. **Dagmar Kettemann:** Data curation; resources; investigation. **Patrick Weydt:** Investigation; data curation; resources; project administration; writing – review and editing. **René Günther:** Writing – review and editing; data curation; resources; project administration; investigation. **Paul Lingor:** Investigation; writing – review and editing; project administration; resources; data curation. **Susanne Petri:** Investigation; writing – review and editing; project administration; resources; data curation. **Jan Christoph Koch:** Data curation; resources; project administration; writing – review and editing; investigation. **Julian Großkreutz:** Investigation; conceptualization; writing – review and editing; project administration; data curation; resources. **Annekathrin Rödiger:** Data curation; resources; project administration; writing – review and editing; investigation. **Petra Baum:** Investigation; writing – review and editing; project administration; resources; data curation. **Andreas Hermann:** Data curation; resources; project administration; writing – review and editing; investigation. **Johannes Prudlo:** Writing – review and editing; data curation. **Matthias Boentert:** Investigation; writing – review and editing; project administration; data curation; resources. **Jochen H. Weishaupt:** Data curation; resources; project administration; writing – review and editing; investigation. **Wolfgang N. Löscher:** Investigation; writing – review and editing; project administration; data curation; resources. **Johannes Dorst:** Data curation; resources; project administration; writing – review and editing; investigation. **Yasemin Koc:** Data curation; validation; investigation; methodology. **Sarah Bernsen:** Project administration; resources; data curation; writing – review and editing; investigation. **Isabell Cordts:** Investigation; writing – review and editing; project administration; data curation; resources. **Maximilian Vidovic:** Data curation; resources; project administration; writing – review and editing; investigation. **Robert Steinbach:** Investigation; writing – review and editing; project administration; data curation; resources. **Moritz Metelmann:** Investigation; writing – review and editing; project administration; resources; data curation. **Vera E. Kleinveld:** Data curation; formal analysis; writing – review and editing. **Jenny Norden:** Data curation; investigation. **Albert Ludolph:** Investigation; writing – review and editing; resources. **Bertram Walter:** Software; formal analysis; investigation; methodology; data curation; supervision. **Peggy Schumann:** Data curation; supervision; project administration; formal analysis; software; methodology; validation; conceptualization; funding acquisition. **Christoph Münch:** Conceptualization; funding acquisition; software; project administration; resources; writing – review and editing. **Péter Körtvélyessy:** Writing – review and editing; data curation; supervision; resources; formal analysis; validation; methodology; conceptualization; investigation. **André Maier:** Conceptualization; investigation; project administration; writing – review and editing; resources; data curation; supervision.

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CONFLICT OF INTEREST STATEMENT

T.M. has received grants, personal fees, non-financial support, and research support from AL-S Pharma, Amylyx, Cytokinetics, Ferrer, Mitsubishi Tanabe, Sanofi, Orphazyme, and served on the advisory boards of Amylyx, Biogen, and ITF Pharma outside of the submitted work. T.M. and C.M. are founders and shareholders of the Ambulanzpartner Soziotechnologie APST GmbH, which makes the mobile application "ALS-App." T.G. has received personal fees from ITF Pharma and served on the advisory boards of Amylyx and ITF Pharma outside of the submitted work. P.W. has served on advisory boards of Biogen, ITF Pharma, and Novartis outside of the submitted work. R.G. has received grants, personal fees, non-financial support, and research support from Biogen and served on the advisory boards of Biogen, Roche, and ITF Pharma outside of the submitted work. P.L. has received consulting fees from AbbVie, Alexion, BIAL, Desitin, ITF Pharma, STADA Pharm, Woolsey Pharmaceuticals, and Zambon outside of the submitted work. He is co-inventor on patents EP 2825175 B1, US 9.980,972 B2 for the use of Fasudil in ALS. J.C.K. has received consulting fees and compensations for talks from Biogen, Roche, and AbbVie and has served on advisory boards for Biogen and Roche. S.P. has received speaker fees, non-financial support, and research support from Biogen, Roche, ALS Pharma, Amylyx, Cytokinetics, Ferrer, ITF Pharma, and Sanofi and served on advisory boards of Amylyx, Biogen, Roche, Zambon, and ITF Pharma outside of the submitted work. A.H. has received funding from the European Social Fonds, the Federal Ministry of Education and Research, and the "Hermann und Lilly Schilling-Stiftung für medizinische Forschung im Stifterverband", honoraria for presentations/advisory boards from Amylyx, Desitin, and ITF Pharma, and royalties from Elsevier Press and Kohlhammer. J.P. has received consulting fees from Hormosan Pharma. M.B. has served on advisory boards for Sanofi, Amicus, and Biogen and has received speaker honoraria from Sanofi, Amicus, ITF Pharma, and Biogen, and financial research support from Sanofi and Löwenstein Medical, all outside of the submitted work. J.D. has received speaker honoraria from Biogen, ITF Pharma, and Zambon. I.C. received personal fees from Biogen and Roche (speaker honoraria and/or participation in advisory boards) outside the submitted work. A.L. has served on advisory boards of Roche, Biogen, Alector, and Amylyx and received compensation from Biologix, German Society of Neurology, Biogen, Springer Medicine, Amylyx, and Streamed Up!, and financial research support from Amylyx, Biogen, Ferrer International, Novartis, Mitsubishi Tanabe, Apellis Pharmaceuticals, Alexion, Orion Pharma, Orphazyme, the European Union, and BMBF. P.K. received consulting fees from Biogen. R.S. has received honoraria for presentations from ITF Pharma outside of the submitted work. A.M. has received personal fees, non-financial support, and research support from ITF Pharma and Zambon outside the submitted work. The other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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