RESEARCH ARTICLE

UCHL1 from serum and CSF is a candidate biomarker for amyotrophic lateral sclerosis

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Abstract

Objective: To identify potential ALS biomarkers in patients and to evaluate their diagnostic performance using cerebrospinal fluid (CSF) and serum. **Method**: We recruited a discovery cohort, comprising 20 ALS patients and 20 controls to screen for potential CSF biomarker, UCHL1, using a Luminex neurodegenerative disease panel. To validate UCHL1's diagnostic performance, we used receiver operating characteristic (ROC) curves to determine the potential for early diagnosis in another cohort comprising 23 CSF and 69 serum ALS samples. Finally, we analyzed its correlation with clinical features. **Results**: We found significantly elevated levels of CSF-derived UCHL1 in both discovery and validation cohorts ($P < 0.05$). ROC curves revealed an AUC of 0.8288, with a sensitivity and specificity of 73.91% and 81.25%, respectively, when the cut-off value for UCHL1 was >291.9 pg/mL. A similar result was observed in the serum cohort, with the ALS group exhibiting significantly higher serum UCHL1 levels than the controls ($P < 0.05$). AUC of the ROC in the serum UCHL1 cohort was 0.7709, with sensitivity and specificity of 61.43% and 79.59%, respectively, when the cut-off value of serum UCHL1 was >15.22 pg/mL. At the early stage CSF and serum UCHL1 were significantly different between ALS patients and controls ($P < 0.05$). Furthermore, serum UCHL1 levels showed a positive relationship with the burden of UMN and LMN dysfunction, albeit with no statistical significance. **Interpretation**: Taken together, our findings suggest that ALS patients exhibit significantly elevated CSF- and serum-derived UCHL1. Moreover, our data warrant that UCHL1 displays good diagnostic performance and provide novel options for ALS early diagnosis. However, its prognostic value needs to be further investigated.

Introduction

Amyotrophic lateral sclerosis (ALS) is a rare and fatal neurodegenerative disorder that affects the upper and lower motor neurons of the brain and spinal cord. The disease mainly manifests as progressive muscle atrophy, weakness, and respiratory failure.[1,2] According to a systematic review of 2019, the global ALS prevalence and incidence were 4.42/100 000 and 1.59/100 000/year respectively.[3] Despite numerous research efforts over the past decades, the mechanisms of ALS pathogenesis remain unclear. Previous studies have suggested that multiple factors, such as neuroinflammation, mitochondrial dysfunction, cytoskeletal dynamics, protein, and RNA

homeostasis, may be contributing to the development and progression of the disease.[1,4] In addition, about 5 ~ 10% of ALS patients reportedly have a family ALS history (fALS),[1,5] whereas 90 ~ 95% are classified as sporadic (sALS) cases. To date, more than 50 potential causative or disease-modifying genes have been identified, with the most common ones being *SOD1*, *C9ORF72*, *FUS*, and *TARDBP*.[4,6] However, the proportion of common disease-causing genes varies among different populations. For example, amplified GGGGCC repeat expansions in the *C9ORF72* gene are the most common in European populations, whereas *SOD1* is the most dominant in Asians. Therefore, genetic biomarkers are limited to only a small percentage of ALS patients. Currently, a lack of

efficient biomarkers, for the entire global population, hampers efforts for early diagnosis and development of treatment therapies against ALS. In fact, the current diagnosis, which is based on clinical evaluation and electrophysiological examinations, poses significant challenges since the time between onset of symptoms to diagnosis is usually delayed by about 1 year in most patients.[7]

Therefore, it is imperative to identify new biomarkers that can increase our understanding of the pathophysiology of ALS, and potentially aid in early diagnosis as well as development of novel drugs against the disease. The present study, therefore, aimed to unravel novel ALS biomarker candidates using a comparative analysis of human CSF and blood samples from symptomatic ALS patients and controls.

Summarily, we selected a panel of candidate neurodegenerative biomarkers, based on our previous research and other published studies. These included Glial fibrillary acidic protein (GFAP),[8,9] DJ1,[10] S100B,[9,11] Amyloid beta 40 (Abeta 40), Amyloid beta 42 (Abeta 42),[9] Transglutaminase 2 (TGM2),[12] α-synulein,[13] Ubiquitin C-terminal hydrolase L1 (UCHL1),[14] and Neuron specific enolase (NSE).[15] We recruited a discovery cohort, comprising 20 ALS patients and 20 patients with other neurological diseases, and screened it for possible biomarkers using the Luminex platform. We then validated the diagnostic performance of the biomarker using CSF and serum cohorts, and further assessed their potential for early diagnosis.

Materials and Method

Sample collection

Patients with ALS were recruited from the Department of Neurology, at the First Centre, Chinese PLA General Hospital, Beijing, between October 2018 and December 2019. Diagnosis was performed by experienced ALS neurologists according to the revised criteria from E1 Escorial. CSF and blood were collected from all patients at first visit, before the final diagnosis was established. Demographic characteristics for the discovery and validation cohorts are listed in Table 1. Briefly, disease duration was defined as the time between symptom onset and sampling, whereas symptom onset was defined as first signs of muscle weakness or dysarthria reported by the patients and subsequently confirmed by examination. Those less than 18 years old were excluded. All patients gave written informed consent, before being included in this study which was approved by the Ethics Committee of Chinese PLA General Hospital.

A total of 20 ALS patients and 20 controls with neurodegenerative disorders were enrolled into the discovery cohort and screened for potential biomarkers. Thereafter,

Table 1. Characteristics of ALS patients and controls from discovery and validation cohorts.

Category	N	Sex (Male/Female)	Age
The discovery cohort	40		
ALS	20	16/4	49.08 ± 9.699
Control	20	15/5	49 ± 12.351
Difference between the groups		$P = 1.000$	$P = 0.986$
The CSF validation cohort	40		
ALS	23	16/7	52.70 ± 10.368
Control	17	10/7	47.06 ± 15.638
Difference between the groups		$P = 0.481$	$P = 0.178$
The Serum validation cohort	118		
ALS	69	51/18	53.13 ± 10.491
Control	49	30/19	54.82 ± 11.418
Difference between the groups		$P = 0.143$	$P = 0.409$

ALS, Amyotrophic lateral sclerosis.

a different group, comprising 69 ALS patients and 49 controls, were enrolled into a validation cohort. Not all patients' CSF and serum samples were obtained. The CSF validation cohort of 23 patients with ALS and 17 controls was therefore evaluated for CSF UCHL1 as ALS biomarker candidate, with the serum validation cohort comprising 69 ALS patients and 49 controls enrolled to determine the performance of serum UCHL1 as a potential ALS biomarker. CSF was collected by lumbar puncture, during diagnostic workup, aliquoted and immediately stored at −80°C until further analysis. Serum was collected from each subject by venipuncture, then centrifuged at 1500 × *g* for 15 minutes within an hour after blood collection. The serum was, thereafter, aliquoted and stored at −80°C until further analysis.

Quantification of CSF analytes

CSF samples in the discovery cohort were analyzed using a Luminex® xMAP Bead Array Platform (Millipore Corporation). Briefly, a selected panel from the Human neurodegenerative disease kit was used to quantify a combination of nine neurological cytokines. The selected panel included the following: Glial fibrillary acidic protein (GFAP), DJ1, S100B, Amyloid beta 40 (Abeta 40), Amyloid beta 42 (Abeta 42), Transglutaminase 2(TGM2), α-synulein, Ubiquitin C-terminal hydrolase L1(UCHL1) and Neuron specific enolase (NSE). These assays were

conducted according to the instructions described on the instrument, and the resulting data imported into the Milliplex analyte v.1.0.0. software for analysis.

Measurement of CSF and serum UCHL1

UCHL1 levels in CSF and serum samples were measured using the ultrasensitive single molecule array(Simoa) Human Ubiquitin C-terminal hydrolase L1 assay, on a Simoa HD-1 Analyer(Quanterix, MA), according to the manufacturer's instructions. Mean variation coefficients of UCHL1 measurements in QC and serum samples were <5% and <20% respectively.

Statistical analysis

Normality distribution was determined with the Shapiro–Wilk test. In non-normally distributed data, the non-parametric Kruskal–Wallis test and the 2 tailed unpaired Mann–Whitney U test were used at a 5% significance level. For multiple comparisons, Dunn post hoc test was performed after the Kruskal–Wallis test to verify the difference between groups at a significance level of 5%. In normally distributed data, differences between ALS patients and the controls were assessed using a two-tailed unpaired *t*-test and *Chi*-square test for continuous and categorical variables respectively. UCHL1's diagnostic performance in CSF and serum samples was visualized by receiver operating characteristic (ROC) curves, with the optimal cut-off calculated using the highest Youden Index. For the optimal cut-off, sensitivity, specificity, and predictive values were calculated at 95% confidence intervals (CI) and the area under the curve (AUC). Values with $P < 0.05$ were regarded statistically significant. Correlations were analyzed by the Spearman correlation test at a 5% significance level. All figures were generated using GraphPad Prism 8.

Results

Patient characteristics

The demographics of ALS patients and controls are summarized in Tables 1 and 2. In the discovery cohort, mean ages of ALS patients and controls were 49.08 ± 9.699 and 49 ± 12.351 ($P = 0.986 > 0.05$) respectively. In the CSF validation cohort, mean ages for ALS patients and controls were 52.70 ± 10.368 and 47.06 ± 15.638 ($P = 0.178 > 0.05$) respectively. Similarly, no significant differences were observed in age between ALS patients and controls in the serum validation cohort (ALS: 53.13 ± 10.491, Control: 54.82 ± 11.418, $P = 0.409$), as well as in sex ($P > 0.05$) between groups and across cohorts(Table 1).

Clinical information for ALS patients is listed in Table 3. Summarily, 20 of 23 patients, in the CSF validation cohort were diagnosed as definite, 2 exhibited probable, and one had possible ALS. Bulbar and the spine were the sites of onset in 4 and 19 cases respectively. Of the 69 ALS patients in the serum validation cohort, 43, 11, and

Table 2. Demographics and clinical data of the disease controls.

No	Disease diagnosis	N	M/F	Age	Serum UCHL1 concentration (pg/mL)	N	M/F	Age	CSF UCHL1 concentration (pg/mL)
				Serum cohort of disease controls				CSF cohort of disease controls	
1	Cervical stenosis	9	7/2	59.2 (47-66)	9.55 (4.97-24.35)	2	2/0	48.3 (39-62)	279.90 (189.83-369.97)
2	Multiple system atrophy	8	4/4	63.37 (48-78)	11.845 (6.37-22.69)	2	0/2	54 (44-64)	265.93 (144.05-387.81)
3	Multiple sclerosis	7	3/4	47 (33-55)	10.55 (2.69-21.69)	3	2/1	53.6 (44-70)	257.57 (229.39-282.21)
4	Polyneuropathy	6	5/1	51 (40-72)	8.86 (3.67-14.51)	3	3/0	50 (37-71)	194.49 (119.42-246.54)
5	Myasthenia gravis	5	3/2	60 (46-77)	16.66 (8.30-21.86)	1	1/0	43	383.25
6	Guillain-Barré Syndrome	4	1/3	45.75 (30-55)	9.89 (3.22-23.68)	1	0/1	30	252.2
7	Radiculopathy, plexopathy	2	1/1	60.5 (49-72)	4.4 (2.44-6.42)	1	0/1	49	254.55
8	Multifocal motor neuropathy	2	2/0	48.5 (39-58)	12.19 (11.82-12.56)	2	1/1	38 (37-39)	235.30 (210.07-260.54)
9	Primary progressive MS	1	1/0	55	16.30	1	1/0	55	266.21
10	Kennedy disease	1	0/1	35	4.26	1	0/1	35	213.31
11	CIDP	1	1/0	60	14.34				
12	Leukoencephalopathy	1	0/1	55	5.79				
13	Progressive supranuclear palsy	1	1/0	67	15.31				
14	Lambert Eaton Myasthenia Syndrome	1	1/0	38	2.17				

Median and ranges are given.

CSF, cerebraospinal fluid; f, female; m, male; MS, multiple sclerosis; CIDP, chronic inflammatory demyelinating polyneuropathy.

Table 3. Clinical features of the CSF and serum validation cohorts.

	The CSF validation cohort			The serum validation cohort		
	ALS	Controls	P	ALS	Controls	P
Cases	23	17		69	49	
Onset site:						
Limb	19	-		58	-	
Bulbar	4	-		11	-	
Diagnosis:						
Definite ALS	20	-		43	-	
Probable ALS	2	-		11	-	
Possible ALS	1	-		13	-	
UCHL1 (Mean ± SEM) pg/mL	362.8 ± 17.59	252.2 ± 19.25	0.0002	24.31 ± 2.681	10.66 ± 0.8733	<0.0001

ALS, Amyotrophic lateral sclerosis.

13 diagnosed as definite, probable, and possible ALS respectively. Bulbar and the spine were sites for onset in 11 and 58 cases, respectively.

Biomarker concentrations in the discovery cohort

Results from the neurodegenerative disease panel,used for screening possible biomarkers on the Luminex platform, revealed no significant changes in concentrations of GFAP, DJ1, S100B, Abeta 40, Abeta 42, TGM2, NSE, and α-synulein in the samples from the discovery cohort between ALS and control groups ($P > 0.05$). However, CSF UCHL1 was significantly elevated in ALS patients (UCHL1 of ALS patients: 422.5 ± 38.1 pg/mL) relative to controls (UCHL1 of controls: 201.2 ± 22.0 pg/mL) ($P < 0.0001$) (Figure 1).

Biomarker concentrations in the validation cohort

Results from the Simoa assay, on 69 ALS patients and 49 controls, revealed a significantly higher mean CSF UCHL1 in 23 ALS patients compared to the 17 controls ($P = 0.0002$) (Figure 2A). Levels of serum UCHL1 were also higher in ALS patients relative to controls ($P < 0.0001$) (Figure 2B). UCHL1 concentrations show a significant correlation in 23 matched CSF and serum ALS samples, with lower UCHL1 concentrations in serum compared with CSF ($r = 0.7709$, 95% CI: 0.5259- 0.8978, $P < 0.0001$) (Figure 3).

ROC analysis of UCHL1 in serum and CSF samples in the validation cohort

ALS patients exhibited significantly higher CSF- and serum-derived UCHL1 than control groups in both discovery and validation cohorts. To further assess the diagnostic potential of this biomarker, ROC curves were employed using UCHL1 concentrations in the CSF and Serum UCHL1 cohorts. The AUC value, of ROC analysis, in the CSF UCHL1 cohort was 0.8288 (95% CI: 0.6978-0.9598). When the cut-off value for CSF-derived UCHL1 was >291.9 pg/mL, we obtained sensitivity and specificity of 73.91% (95% CI: 53.53-87.45) and 81.25% (95% CI: 56.99-93.41) ($P = 0.0006$) respectively (Figure 2C). In the serum-derived UCHL1 cohort, the curve AUC was 0.7709 (95% CI 0.6881-0.8536). Applying the cut-off value of serum UCHL1 > 15.22 pg/mL, resulted in sensitivity and specificity values of 61.43% (95% CI: 49.72-71.95) and 79.59% (95% CI: 66.36-88.52) ($P < 0.0001$) respectively (Figure 2D).

Correlation with clinical parameters of ALS

The time taken from onset of symptom to diagnosis represents a significant delay of about 1 year in most patients. It is, therefore, necessary to determine the potential of using UCHL1 for early ALS diagnosis. To evaluate the UCHL1 concentrations at an earlier stage of the disease, a subset of patients with ALS with a symptom duration below or equal to 6 months were selected as it represents the first 25th percentile of the symptom duration of our total ALS cohort. The 6-month time point was set as the cut-off point, and the ALS patients were divided into two subgroups: ALS ≤ 6 months and ALS > 6 months. Results indicated that CSF- and serum-derived UCHL1 levels were significantly higher in both the stage of ALS ≤ 6 months and the ALS > 6 months, relative to controls ($P < 0.0001$) (Figure 4A,B). However, no significant differences($P > 0.05$) were observed in UCHL1 levels between the early stage of ALS ≤ 6 months and the stage of ALS > 6 months. Unlike CSF UCHL1, serum UCHL1 inversely correlated with the symptom duration($r = -0.5184$, $P = 0.0113$) (Figure 4C).

Analysis of the correlation in affected regions, using an aberrant EMG or clinical LMN involvement, resulted in no significant differences between serum-derived UCHL1 concentrations and the extent of upper and lower motor neuron degeneration. Patients with 0 and 1 regions were placed into group 1, whereas those with 3 and 4 affected regions were grouped into group 3. Serum UCHL1 increased proportionally, with the number of affected regions, which showed a trend toward higher UCHL1 levels when more regions were involved, although no statistically significant correlation was detected (Figure 5). We did not analyze the relationship between CSF UCHL1 levels and the number of affected regions, owing to a limited number of CSF cases in the validation cohort and also because the majority of this cohort affected at least 3 regions.

Discussion

In the current study, we used a panel associated with neurodegenerative disease to screen for potential biomarkers in a discovery cohort. Our results revealed significantly higher UCHL1 levels in ALS patients than that in the control group comprising subjects with other neurodegenerative diseases. We then evaluated UCHL1's diagnostic performance in CSF and serum, collected from ALS patients, and analyzed its potential for early diagnosis. To the best of our knowledge, this is the first study describing quantification UCHL1 levels in both CSF and serum.

Our results indicated that CSF UCHL1 is a promising biomarker for ALS diagnosis, consistent with some previous studies.[14,16] Specifically, we found significantly elevated CSF UCHL1 in both discovery and validation
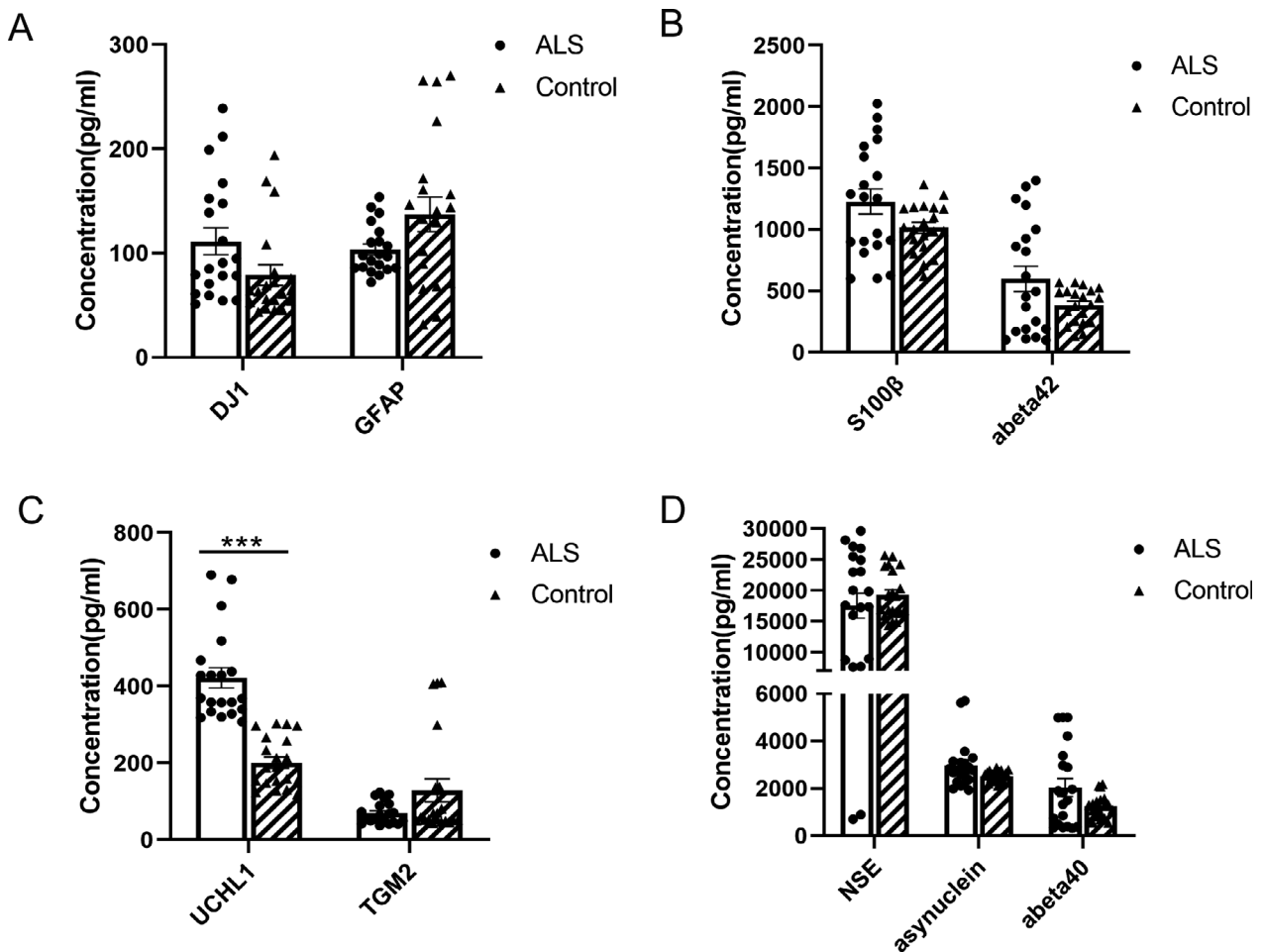

Figure 1. Biomarker levels in the discovery cohort. ALS (n = 20) and controls (n = 20). (A) Concentrations of Glial fibrillary acidic protein (GFAP) and DJ1 between ALS and controls ($P > 0.05$). (B) Levels of S100B and Amyloid beta 42 (Abeta 42) between ALS and controls ($P > 0.05$). (C) Levels of Transglutaminase 2 (TGM2) and Ubiquitin C-terminal hydrolase L1 (UCHL1) between ALS and controls, TGM2 $P > 0.05$, UCHL1 $P < 0.05$. (D) Concentrations of α-synulein, Neuron specific enolase (NSE) and Amyloid beta 40 (Abeta 40) between ALS and controls ($P > 0.05$). Values are Means ± SEM.
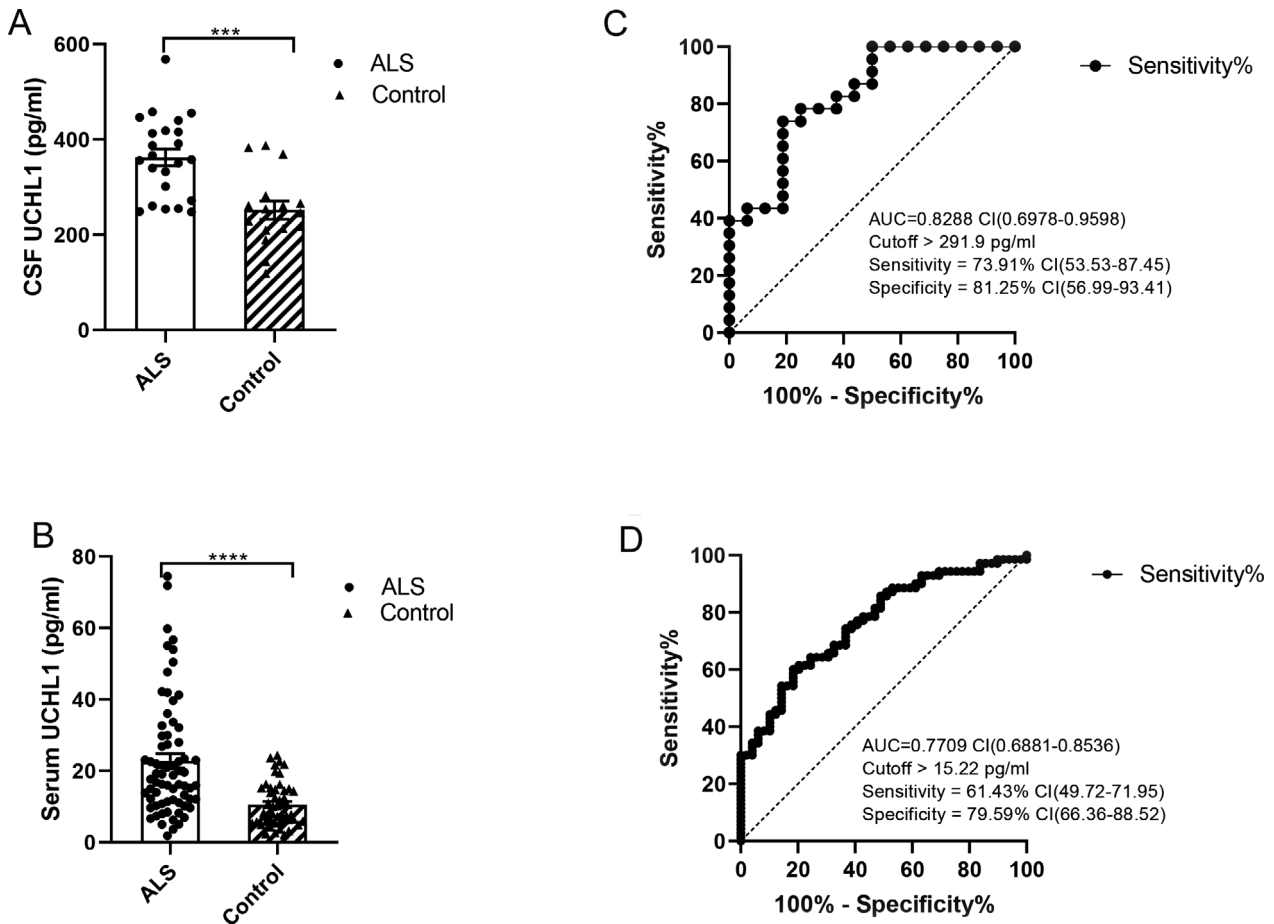
Figure 2. Performance characteristics of UCHL1 in the CSF and serum. (A) Levels of UCHL1 between ALS and controls in the CSF validation cohort ($P < 0.05$). (B) Levels of UCHL1 between ALS and controls in the serum validation cohort ($P < 0.05$). Values are Means ± SEM. Receiver operating characteristic (ROC) analysis of CSF and serum UCHL1 in amyotrophic lateral sclerosis (ALS) relative to controls in the validation cohort. (C) ROC curve of CSF UCHL1 for discriminating between ALS patients and controls. (D) ROC curve of serum UCHL1 for discriminating between ALS patients and controls. AUC = area under the curve; CI = confidence interval.
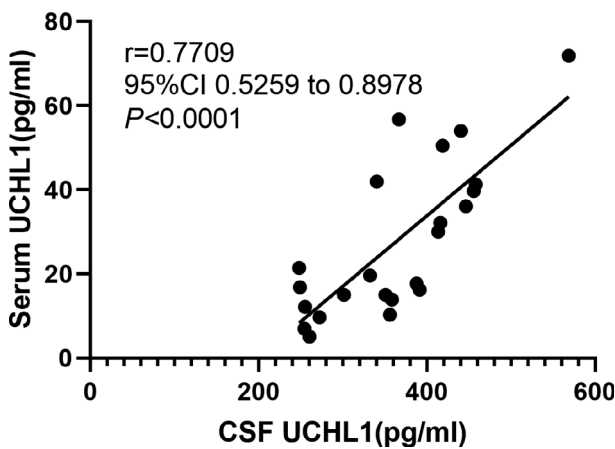

Figure 3. Correlation of matched CSF and serum UCHL1 concentrations. UCHL1 concentrations correlated significantly between matched CSF and serum samples in patients with ALS.

cohorts. ROC curves revealed a high AUC value for CSF UCHL1, indicating that this biomarker has a good ability to distinguish ALS patients from controls. Similarly, good sensitivity and specificity were obtained, when the cut-off value for the CSF UCHL1 was > 291.9 pg/mL. This observation was consistent in the serum cohort, where significantly higher UCHL1 levels were obtained in the ALS than the control group. A high AUC value, as well as good sensitivity and specificity, indicated its ability as a potential biomarker for ALS diagnosis.

UCHL1 is exclusively expressed in the brain, and is estimated to account for $1 \sim 5\%$ of all neuronal proteins.[17] Reports suggest that it functions as a deubiquitinating enzyme, a ubiquitn ligase and a mono-Ub stabilizer, and plays an important role in the ubiquitin-proteasome pathway (UPP) as well as maintenance of the nervous system.[18] Patients with mutations in the *UCHL1*
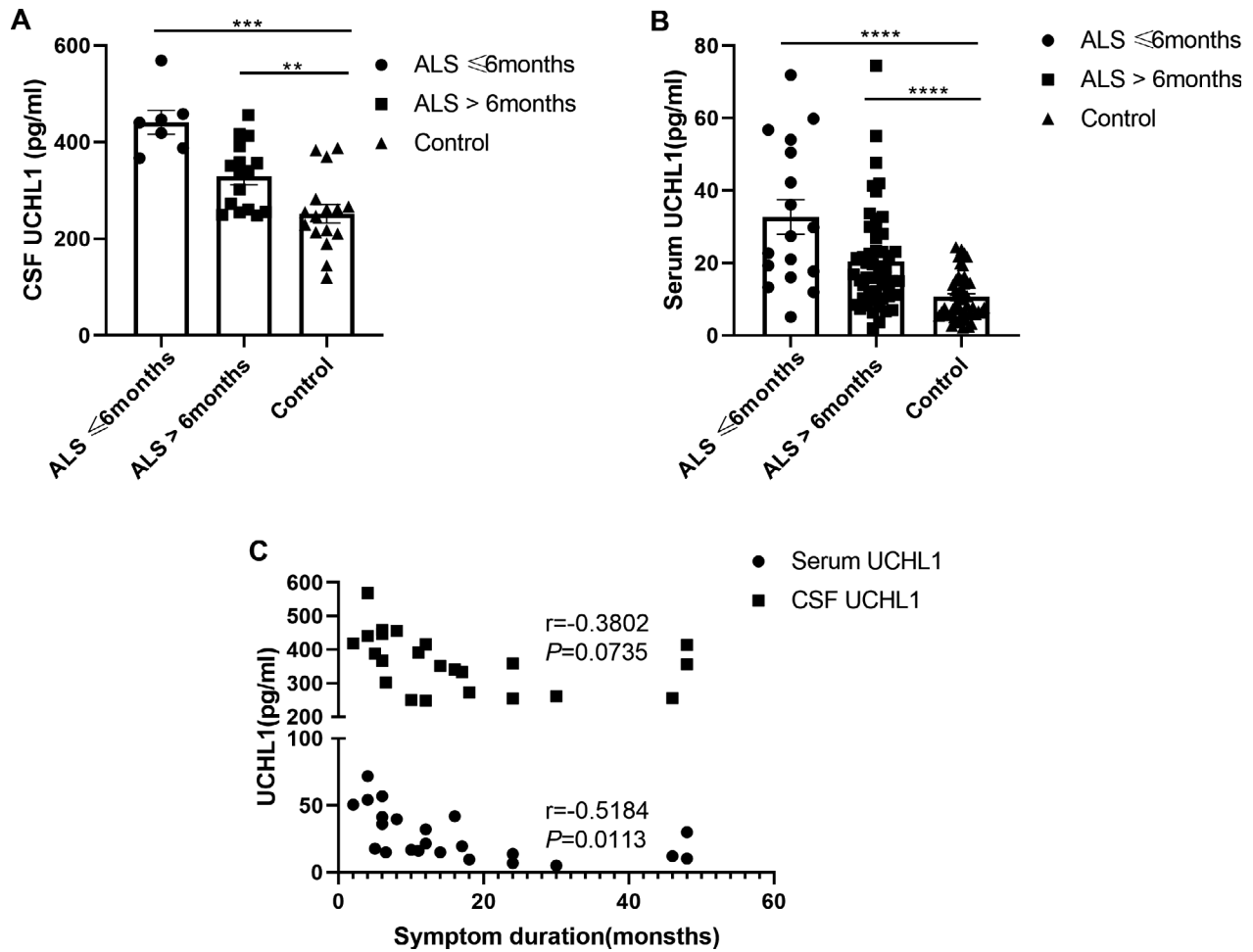
Figure 4. The diagnostic potential of UCHL1 for early diagnosis based on the validation cohorts. We set a 6-month time, from onset to diagnosis, as the point for dividing our ALS patients into 2 different groups. Kruskal-Wallis test with Dunn post hoc correction for multiple testing was used for all comparisons. (A) Different levels of CSF UCHL1 among different diagnosis stages of ALS and controls. (B) Concentrations of serum UCHL1 among different diagnosis stages of ALS and controls. (C) Unlike CSF UCHL1, serum UCHL1 concentrations inversely correlated with symptom duration.

reportedly manifest a movement syndrome.[18] In fact, these mutations have been associated with neurodegenerative diseases,[19-23] including Parkinson's disease (PD),[20,23] Alzheimer's disease (AD),[22] and spastic paraplegia.[21] Recent studies have demonstrated that UCHL1 could be a potential biomarker for discriminating the Parkinson's disease, as well as reflecting disease progression stages and motor severity in this disease.[24,25] Although the direct role of UCHL1 in pathogenesis of PD and AD remains unclear, initial findings from studies on neurodegenerative diseases suggest that UCHL1 could be involved in neurodegeneration of ALS.

This hypothesis has been supported by findings from studies describing differential gene expression and protein analysis in ALS patients and animal models. For example, Recabarren-Leiva et al.[26] systematically analyzed expression profiles of different genes in the motor cortex and spinal cord, and found that *UCHL1* was down-regulated in sALS subjects. These findings were consistent with proteomics profiles of postmortem spinal cord tissues in ALS patients.[16] Although studies focusing on Alternative Polyadenylation (APA) in ALS patients have not reported widespread changes, *UCHL1* unexpectedly shows that the 3' UTR lengthening interacts with TARDBP.[27] Therefore, mutations in *TARDBP* may affect expression and function of UCHL1, which may be part of the pathology mechanism in fALS. Additionally, UCHL1 plays an important role maintaining axonal health and stability.[28] A study, using a mouse model, showed that lack of UCHL1 results in axonal degeneration and neuronal death.[29] UCHL1 is expected to be released into CSF reflecting the axonal and neuron degeneration status, in a similar fashion to the
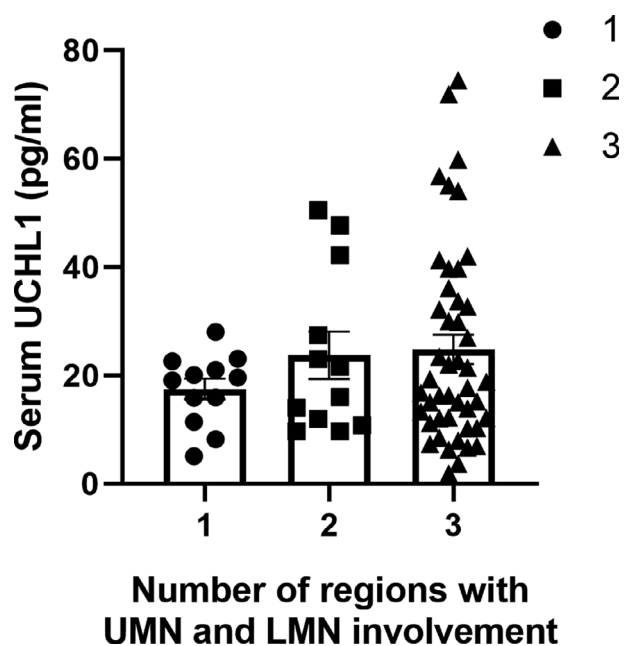
Figure 5. Correlation between Serum UCHL1 levels with the extent of upper motor neuron (UMN) and lower motor neuron (LMN) involvement. To analyze regions with an aberrant EMG or clinical LMN involvement, patients with 0 and 1 regions were categorized into group 1, whereas those with 3 and 4 affected regions were put into group 3. Kruskal-Wallis test with Dunn post hoc correction for multiple testing was used for all comparisons.

neurofilament light in ALS CSF,[30] indicating that CSF UCHL1 in ALS increases. Our data indeed reflected that CSF UCHL1 in ALS patients would be significantly elevated compared with controls. To better evaluate UCHL1 diagnostic performance, we would explore the relationship between UCHL1 and the extent of LMN and UMN dysfunction. However, considering a limited ALS CSF samples, we actually analyzed the correlation in serum samples.

Because UCHL1 is generally highly expressed in the brain,[31] and lack of evidence on UCHL1 expression in red and white blood cells, or platelets, means that the source of elevated UCHL1 in the peripheral blood remains unclear. It has been hypothesized that it could be released from CSF into the blood circulation through impaired blood-brain barriers involved during progression of neurodegenerative diseases. Besides, our data showed that serum UCHL1 levels correlated well with CSF UCHL1 concentrations. Therefore, we explored the correlation between ALS serum UCHL1 and disease duration. Our data displayed serum UCHL1 levels would increase with the number of affected regions in which UMN and LMN were involved, albeit with no statistical significance. This warrants the further evidence to demonstrate UCHL1's good diagnostic performance and

correlation with clinical features, but also reflects our study's limitation that is failure to carry out longitudinal measurements. Therefore, further study would be required to validate our findings.

It's well known that currently ALS diagnosis relies on clinical evaluation and electrophysiological examinations, which mostly result in delayed diagnosis. So it's necessary to evaluate the potential for UCHL1 to be used for early diagnosis targeting CSF and serum. The disease duration of 6 months, as the first 25th percentile of the symptom duration of our ALS cohort, was set as the key point for assessing the ability for early ALS diagnosis. Data revealed that both CSF and serum UCHL1 levels in ALS patients below or equal to 6 months were significantly elevated compared with controls, indicating that UCHL1 may provide additional value for early diagnosis of ALS. Furthermore, levels of serum UCHL1 were higher in ALS patients than in controls and decreased with disease duration, which is consistent with patterns of ALS *UCHL1* gene and protein expression, suggesting a better reflection of motor neuron damage.

To our knowledge, this is the first study reporting UCHL1 levels in blood and CSF samples from ALS patients. We now define cut-offs for serum and CSF UCHL1, which allowed us to investigate its diagnostic performance. Moreover, we also warrant the evidence that ALS patients with a short symptom duration display both CSF and serum increased UCHL1 concentrations. Although the study included a certain number of ALS patients, we acknowledge that a limited number of CSF samples and controls could have a potential effect on the results. In addition, we did not explore UCHL1's prognostic value in ALS patients, as well as its correlation with survival time. Further studies, using a larger longitudinal ALS patient cohort and disease controls, are required to validate our findings.

Acknowledgment

Conflict of Interest

All authors read and approved the final manuscript. They declare no conflicts of interest.

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