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β-synuclein in cerebrospinal fluid as a potential biomarker for distinguishing human prion diseases from Alzheimer's and Parkinson's disease

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Abstract

Background β-synuclein (β-syn), mainly expressed in central nerve system, is one of the biomarkers in cerebrospinal fluid (CSF) and blood for synaptic damage, which has been reported to be elevated in CSF and blood of the patients of prion diseases (PrDs).

Methods We analyzed 314 CSF samples from patients in China National Surveillance for CJD. The diagnostic groups of the 223 patients with PrDs included sporadic Creutzfeldt-Jacob disease (sCJD), genetic CJD (gCJD), fatal familial insomnia (FFI) and Gerstmann-Straussler-Scheinker (GSS). 91 patients with non-PrDs comprised Alzheimer's disease (AD), Parkinson's disease (PD), viral encephalitis (VE) or autoimmune encephalitis (AE) were enrolled in the control groups. The CSF β-syn levels were measured by a commercial microfluidic ELISA. The Mann–Whitney U test and Kruskal–Wallis H test were employed to analyze two or more sets of continuous variables. Multiple linear regression was also performed to evaluate the factors for CSF β-syn levels. Receiver operating characteristics (ROC) curves and area under the curve (AUC) values were used to assess the diagnostic performance of β-syn.

Results The median of β-syn levels (2074 pg/ml; IQR: 691 to 4332) of all PrDs was significantly higher than that of non-PrDs group (504 pg/ml; IQR: 126 to 3374). The CSF β-syn values in the cohorts of sCJD, T188K-gCJD, E200K-gCJD and P102L-GSS were remarkably higher than that of the group of AD + PD, but similar as that of the group of VE + AE. The elevated CSF β-syn in sCJD and gCJD cases was statistically associated with CSF 14-3-3 positive and appearance of mutism. ROC curve analysis identified satisfied performance for distinguishing from AD + PD, with high AUC values in sCJD (0.7640), T188K-gCJD (0.8489), E200K-gCJD (0.8548), P102L-GSS (0.7689) and D178N-FFI (0.7210), respectively.

Conclusion Our data here indicate that CSF β-syn is a potential biomarker for distinguishing PrDs (gCJD, sCJD and GSS) from AD and PD, but is much less efficient from VE and AE. These findings have critical implications for early diagnosis and monitoring of synaptic integrity in prion diseases.

Keywords β-synuclein, Cerebrospinal fluid, Human prion diseases

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Background

Prion diseases (PrDs) are a group of fatal and transmissible neurodegenerative disorders of humans and animals. The infectious agent is known as prion, a pathogenic misfolded and aggregated protein (PrP^{Sc}), sharing the same amino acid sequence as a host surface protein (PrP^{C}) [1, 2]. The main neuropathological changes of PrDs involve spongiform degeneration, synaptic alterations, brain inflammation and neuronal death [3]. Human PrDs comprises sporadic, genetic, and acquired forms. The most common form is sporadic Creutzfeldt-Jakob disease (sCJD), accounting for more than 85% of all PrDs. 10%~15% of PrDs are predominantly inherited involving in different mutations in prion protein (*PRNP*) gene, including genetic CJD (gCJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). Less than 1% of prion diseases are acquired, including Kuru, iatrogenic CJD (iCJD) and variant CJD (vCJD) [4]. The accurate diagnosis of patients with PrDs still remains a challenge. Because of the variable clinical presentations that often overlap with those of other rapidly progressive dementias (RPDs) [5], the definite diagnosis depends on the brain postmortem or biopsy with either neuropathological vacuolation or PrP^{Sc} deposit. In the past decades, many cerebrospinal fluid (CSF) proteins, e.g., protein 14-3-3, total tau and neurofilament light chain protein (NfL), etc., have shown significance in the diagnostics of PrDs [6, 7], although there is still controversial and limited utility in discriminating PrDs from other RPDs [8–10]. The developments of two types of in vitro protein amplification techniques, protein misfolding cyclic amplification (PMCA) and real-time quaking induced conversion (RT-QuIC), have revealed the great advantage in prion research. In particular, RT-QuIC based on the specimen CSF, skin or olfactory mucosa, has shown good performance in the diagnosis of PrDs [11–13]. However, RT-QuIC is still not available in some laboratories due to the complicate process and the special equipment, which limits its widespread clinical application [14]. Therefore, the search for diagnostic biomarkers in different human specimens for PrDs is an ongoing endeavor.

Beta-synuclein (β-syn), consisting of 134 amino acids, is a 14 kDa presynaptic protein expressed in the neurons of the central nervous system (CNS) and enriched in the neocortex and hippocampus [15]. It shows multiple functions including inhibition of α-synuclein (α-syn) aggregation, regulation of synaptic function, mediation of apoptosis, and involvement in protein degradation pathways [16]. Synapses are the sites of signal transmission and memory formation in the brain and synapse degeneration is the early stage of neurodegenerative disorders [17, 18]. β-syn can be measured by the methodologies of mass spectrometry (MS) and ELISA or digital ELISA [19]. Compared to α-syn, β-syn is more CNS-specific and can be a promising CSF and blood biomarker for synaptic damage. Recently, elevated β-syn has been described in CSF and blood of the patients with PrDs and Alzheimer's disease (AD) [20–22]. The clinical value of β-syn in CSF and/or blood needs the observation of multicenter and larger sample size.

In this study, the CSF β-syn levels in 223 Chinese patients with five types of PrDs, including probable sCJD, T188K-gCJD, E200K-gCJD, P102L-GSS and D178N-FFI, from China National Surveillance for CJD (CNS-CJD) were individually measured using a microfluidic ELISA technique, together controlled with 91 cases of non-PrDs who were clinically diagnosed as AD, Parkinson's disease (PD), viral encephalitis (VE) or autoimmune encephalitis (AE). The diagnostic performance of CSF β-syn was assessed with receiver-operating characteristic (ROC) curve analysis. Furthermore, we investigated the potential correlation between CSF β-syn values, demographic and clinical parameters.

Materials and methods

Study participants

All cases enrolled in this study were from the National Surveillance for CJD (CNS-CJD). The case diagnosis and classification were conducted according to the Diagnostic Criteria for CJD issued by the Chinese National Health Commission. The inclusion criteria also required the participants having intact information of demographics, neurology, clinical examination, and laboratory test. Additionally, all enrolled sCJD cases were positive in either CSF or skin RT-QuIC, whilst all gPrD cases contained positive genetic mutation in *PRNP* verified by sequencing. Individuals lacking demographic information or CSF sample volume less than 30 μl were excluded from the study. Finally, 223 patients diagnosed with PrDs were included in the PrDs group, including sCJD ($n=86$), T188K-gCJD ($n=58$), E200K-gCJD ($n=32$), P102L-GSS ($n=14$), and D178N-FFI ($n=33$). For comparison, 91 cases were enrolled as the control group (non-PrDs) who did not fulfill the diagnostic criteria for PrDs and have had additional diagnosis afterwards, including AD ($n=8$), PD ($n=9$), VE ($n=18$), and AE ($n=56$). *PRNP* sequencing was conducted on all PrD and non-PrD cases. Among 314 enrolled cases, 306 were 129 M/M, 7 were 129 M/V and 1 were 129 V/V. Due to the inaccessibility of brain tissue, we did not perform neuropathological examination.

CSF samples

The CSF samples were obtained from the local hospitals and subsequently transferred to the central laboratory of

CNS-CJD at China CDC. All CSF specimens were free of blood contamination and underwent centrifugation at 2,000 rpm for 1 min. The CSF specimens were aliquoted and stored at −80 °C until analysis. The use of these stored samples was approved by the Ethics Committee of the National Institute for Viral Disease Control and Prevention, China CDC, and personal information was limited to age, gender, clinical symptoms and examination results.

Simple plex™ assay for β-synuclein

30 µl of CSF sample from each case was applied for β-syn detection using a commercially available microfluidic Simple Plex cartridge (SPCKB-PS-010127) on an automated ELISA immunoassay system (Bio-techne, Ella, USA) according to the manufacturer's instructions. The Ella instrument automates the entire immunoassay workflow, including reagent addition, incubation, washing, and detection. CSF samples are diluted 1:2 with diluent SD13 (#896098). For users, we only need to scan the barcode on the Simple Plex cartridge and load the diluted samples, and then insert the cartridge into the Ella instrument. The Simple Plex cartridge features a unique microfluidic design with multiple parallel channels, each containing three glass nano reactors (GNRs). These GNRs are coated with specific capture antibodies that bind to β-syn in the sample. According to the specification sheet, the limit of detection (LOD), lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) for human β-syn were 1.83 pg/ml, 7.96 pg/ml and 4856 pg/ml, respectively.

Statistical analysis

The data were statistically analyzed using GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA) and SPSS 25.0 statistical software (IBM, Armonk, NY., USA). Descriptive statistics were expressed as median (IQR, interquartile range) for continuous variables and as percentage (%) for categorical variables. The Mann–Whitney U test and Kruskal–Wallis H test were employed to analyze two or more sets of continuous variables after assessing normal distribution with the Shapiro–Wilk test. Multiple linear regression Analysis was utilized for multivariate analysis. In this model, factors potential influencing CSF β-syn results are considered as independent variables, while CSF β-syn is designated as the dependent variable. Receiver operating characteristics (ROC) curves and area under the curve (AUC) values were used to assess the diagnostic performance of β-syn between the group of PrDs and non-PrDs, as well as between individual subgroups of types of PrDs and different sorts of diseases in non-PrDs. AUC is a key indicator of the overall performance of the ROC model, independent of the cut-off. Higher AUC values mean better model classification. The B value, or logistic regression coefficient, indicates the impact and direction of an independent variable on a dependent variable. A positive B value means that as the predictor variable increases, the log-odds of the outcome also increase, suggesting a higher probability of the event occurring. All tests were two-sided, with statistical significance set at $p < 0.05$ and p-values were denoted as ****($p < 0.0001$), ***($p < 0.001$), **($p < 0.01$), *($p < 0.05$), or NS (not significant).

Results

Demographical and clinical characteristics

The main demographic and clinical features of 223 Chinese PrD cases with various types and 91 non-PrD cases were summarized in Table 1. The medians of onset ages of the patients were 64 y in sCJD, 62 y in T188K-gCJD, 57 y in E200K-gCJD, 49 y in P102L-GSS, 55 y in D178N-FFI, respectively. Progressive dementia was overwhelming recorded in the PrD patients. The other four major clinical symptoms were also frequently noticed in PrD cases without significant difference among various types of PrDs. CSF 14-3-3 positivity was identified in approximately 65 to 70% of sCJD, T188K- and E200K gCJD patients, 50% of P102-GSS cases and 33.3% of D178N-FFI. PSWC on EEG was frequently noticed in sCJD (70.8%) and E200K-gCJD (61.9%), less frequent in T188K-gCJD (38.6%) and P103L-GSS (12.5%), while undetectable in D178N-FFI (0%). *PRNP* sequencing revealed a predominant polymorphism of 129 M/M (306 cases, 97.5%), whilst much less of 129 M/V (7 cases, 2.2%) and 129 V/V (1 case, 0.3%). The clinical durations of different types of PrD cases were short in sCJD, T188K- and E200K-gCJD (median from 3 to 5 months), but obviously long in D178N-FFI (10 months). The cohort of non-PrDs consisted of 8 cases of AD, 9 cases of PD, 18 cases of viral encephalitis (VE) and 56 cases of autoimmune encephalitis (AE). The medians of the onset ages were 64 y in the group of AD + PD and 58 y in that of VE + AE. Besides of dementia, the other four clinical symptoms were less observable in non-PrD patients compared to sCJD. The positive rates of CSF 14-3-3, PSWC on EEG, and abnormalities on MRI in the cohort of non-PrDs were markedly lower than that of sCJD and gCJD, particularly PSWC on EEG.

CSF β-syn levels in various cohorts of PrDs and non-PrDs

Compared to the median of CSF β-syn levels (504 pg/ml; IQR: 126 to 3374) in the group of non-PrDs, the median of β-syn (2074 pg/ml; IQR: 691 to 4332) of all PrDs was remarkably higher, showing significant difference (Fig. 1A). Subsequently, β-syn values were calculated separately in various types of PrDs and non-PrDs.

Table 1 Demographic and clinical characteristics of the patients with PrDs and non-PrDs

Characteristics	PrDs					non-PrDs	
	sCJD	T188K-gCJD	E200K-gCJD	P102L-GSS	D178N-FFI	AD + PD	VE + AE
No	86	58	32	14	33	17	74
Gender (M/F)	46/40	34/24	11/21	6/8	18/15	7/10	46/28
Median age at onset (y, IQR)	64 (55–69)	62 (56–68)	57 (49–66)	49 (43–59)	55 (43–63)	64 (56–70)	58 (47–69)
CSF 14-3-3 Positive/Total no. (%)	56 (65.1)	40 (69.0)	20/31 (64.5)	7 (50.0)	11 (33.3)	8 (47.1)	35/71 (49.3)
129 MM of *PRNP*/Total no. (%)	82 (95.3)	58 (100.0)	31 (96.9)	14 (100.0)	33 (100.0)	16 (94.1)	72 (97.3)
129 MV of *PRNP*/Total no. (%)	3 (3.5)	0 (0.0)	1 (3.1)	0 (0.0)	0 (0.0)	1 (5.9)	2 (2.7)
129 VV of *PRNP*/Total no. (%)	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
PSWC in EEG (%)	17/24 (70.8)	17/44 (38.6)	13/21 (61.9)	1/8 (12.5)	0 (0.0)	1/17 (5.9)	1/47 (2.1)
MRI abnormal change/Total no. (%)	70/84 (83.3)	46/57 (80.7)	29 (90.6)	13 (92.9)	7/30 (23.3)	4/16 (25.0)	24/69 (34.8)
Progressive dementia/Total no. (%)	85 (98.8)	58 (100.0)	32 (100.0)	13 (92.9)	33 (100.0)	16 (94.1)	74 (100.0)
Myoclonus no. (%)	53 (61.6)	34 (58.6)	22 (68.8)	5 (35.7)	17 (51.5)	2 (11.8)	27 (36.5)
Visual or cerebellar disturbance no. (%)	67 (77.9)	42 (72.4)	28 (87.5)	9 (64.3)	21 (63.6)	5 (29.4)	12 (16.2)
Pyramidal or extrapyramidal dysfunction no. (%)	76 (88.4)	44 (75.9)	29 (90.6)	12 (85.7)	25 (75.8)	8 (47.1)	26 (35.1)
Akinetic Mutism no. (%)	34 (39.5)	30 (51.7)	16 (50.0)	4 (28.6)	5 (15.2)	4 (23.5)	10 (13.5)
Individuals with survival time no. (%)	37 (43.0)	25 (43.1)	4 (12.5)	2 (14.3)	6 (18.2)	1 (5.9)	2 (2.7)
Survival time (month, IQR)	3 (2.0–3.5)	5 (4.0–6.0)	4.5 (3.3–9.5)	NA	10 (6.3 ~ 15.0)	14	NA

Demographic data are expressed as percentage (%), while biomarkers values and survival time as median (IQR)

Abbreviations: *PWSC* periodic sharp wave complexes, *EEG* electroencephalogram, *MRI* magnetic resonance imaging, *CSF* cerebrospinal fluid, *IQR* interquartile range, *N/A* not applicable

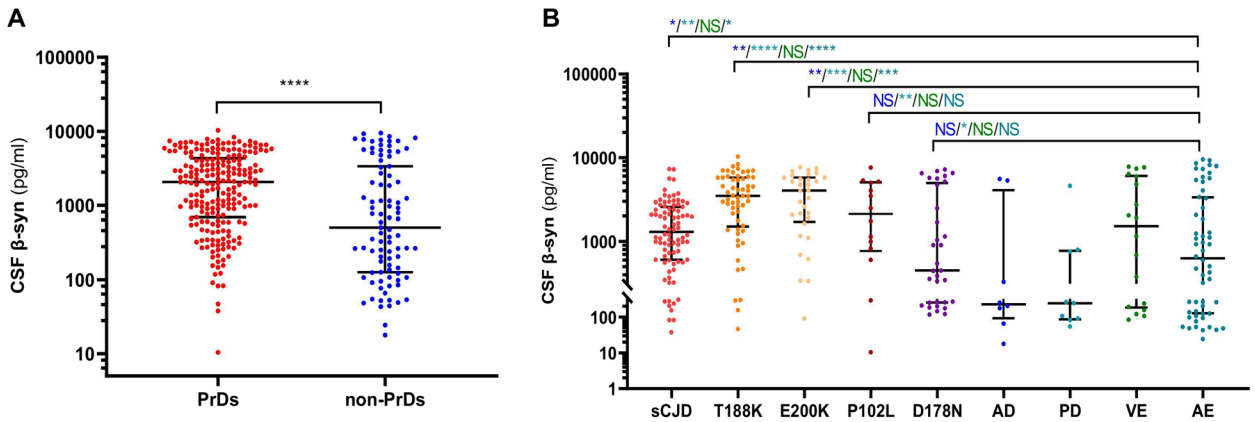

Fig. 1 CSF β-syn levels in the cohorts of various diseases. **A** Comparison between the groups of total PrDs and non-PrDs. **B** Comparison among the groups of different PrDs (sCJD, T188K-gCJD, E200K-gCJD, P102L-GSS, D178N-FFI) and non-PrDs (AD, PD, VE, AE). β-syn values are presented as log (pg/ml); median, and interquartile range are shown for the biomarker levels. The *p*-values for the Mann–Whitney test are indicated above. The statistical outcomes of the corresponding PrDs, as compared to AD, PD, VE, AE, are represented by dark blue, sky blue, green, and cyan colors, respectively

As shown in Fig. 1B and Supplementary Table 1, the values of CSF β-syn of different PrDs and non-PrDs revealed different profiles, showing notably high median values in the groups of T188K-gCJD (3506.5 pg/ml; IQR: 1500.3 to 5822.0) and E200K-gCJD (4012.5 pg/ml; IQR: 1707.0 to 5765.0), followed by P102L-GSS (2114.5 pg/ml; IQR: 767.5 to 5043.3) and sCJD (1304.0 pg/ml; IQR: 605.5 to 2581.5), whilst lowest value in D178N-FFI (448.0 pg/ml; IQR: 255.5 to 4941.5). In non-PrDs cohort, the β-syn median values in the patients of VE (1527.5 pg/ml; IQR: 184.3 to 6058.3) and AE (628.5 pg/ml; IQR: 128.5 to 3349.3) were higher than those of AD (228.5 pg/ml; IQR: 93.7 to 4073.5) and PD (245.0 pg/ml; IQR: 87.1 to 777.5). Statistical assays identified the significances in β-syn values of two types of gCJD with AD, PD and AE, sCJD with AD and PD, P102L-GSS with PD, respectively,

whereas no significance of D178N-FFI with any types of non-PrDs. Additionally, there was no statistical difference in β-syn values of VE cases with any types of PrD patients. The median intervals from onset to sampling in the cohorts of total PrDs (3 months; IQR: 2 to 6) and total non-PrDs (3 months; IQR: 1 to 9) were quite comparable. For a particular disease, the intervals in the groups of P102L-GSS, D178N-FFI, AD and PD were relatively longer. Spearman correlation analysis did not identify a significant correlation between the β-syn level and the interval from onset to CSF sampling, not only in the context of total PrD or total non-PrD cases, but also in every particular disease (Supplemental Table 2).

Associations of CSF β-syn values with some demographic and clinical items in the patients of sCJD and gCJD

The potential associations of CSF β-syn levels in 176 sCJD and gCJD cases with some clinical elements were analyzed, including 86 sCJD cases, 58 T188K-gCJD and 32 E200K-gCJD. As shown in Table 2, univariate analysis identified the significantly higher β-syn level in the patients with CSF 14–3-3 positive ($P=0.000$), with appearances of myoclonus ($P=0.015$) and mutism ($P=0.003$). Multivariate analysis confirmed the significances in the factors of CSF 14-3-3 (B = 1462.93, $P=0.000$) and mutism (B = 907.34, $P=0.008$). No clear relationship was figured out between CSF β-syn values with other tested factors, such as gender, the onset age, PSWC on EEG, abnormalities on MRI, appearances of other major neurological manifestations. Although the median values of CSF β-syn showed an increasing trend along with the prolongation of clinical duration, no statistical difference was observed among patients with survival times of less than 3 months, between 3 and 5 months, and longer than 5 months.

Table 2 Univariate and multivariate analysis of CSF β-syn levels in sCJD and gCJD groups

Factors		No	β-syn median (pg/ml, IQR)	Univariate analysis		Multivariate analysis		
				Test's statistic	*p*-value	B (95% CI)	t	*p*-value
Gender	Male	85	2366 (990.00–4141.50)	3736.0	0.697[a]	NA	NA	NA
	Female	91	2191 (730.00–4332.00)					
Median age at onset (year)	< 50	20	1215.5 (600.25–2478.50)	3.4	0.186[b]	NA	NA	NA
	50 ~ 70	130	2591 (994.25–4374.25)					
	> 70	26	2014.5 (706.50–4801.25)					
WB CSF 14-3-3	Negative	59	1335 (537.00–2612.00)	2031.5	0.000[a]	1462.93 (792.59–2133.27)	4.308	0.000
	Positive	116	2973.5 (1139.25–5523.25)					
EEG	Normal	42	3137 (888.25–5589.00)	972.5	0.905[a]	NA	NA	NA
	PSWC	47	2755 (1217.00–5756.00)					
MRI	Normal	29	2109 (497.00–3002.50)	1666.5	0.087[a]	NA	NA	NA
	Abnormal	144	2441.5 (992.25–4531.00)					
Myoclonus	No	67	1538 (606.00–3098.00)	2849.5	0.015[a]	260.34 (–424.57–945.26)	0.750	0.454
	Yes	109	2724 (1104.50–4939.50)					
Visual or cerebellar disturbance	No	39	2498 (889.00–4158.00)	2567.0	0.710[a]	NA	NA	NA
	Yes	137	2166 (957.50–4228.50)					
Pyramidal or extrapyramidal dysfunction	No	27	1989 (606.00–4078.00)	1802.0	0.390[a]	NA	NA	NA
	Yes	149	2385 (976.00–4245.00)					
Akinetic Mutism	No	96	1949.5 (732.50–3123.50)	2838.0	0.003[a]	907.34 (244.77–1569.91)	2.703	0.008
	Yes	80	2993 (1111.00–5719.50)					
Survival time (month)	< 3	16	1447 (909.50–2128.00)	4.5	0.104[b]	NA	NA	NA
	3 ~ 5	38	2057 (1154.25–4771.00)					
	> 5	12	3143.5 (977.25–5753.25)					

CSF β-syn levels are shown as median (interquartile range). Data of Univariate analysis were tested using the Mann–Whitney U test and Kruskal–Wallis H test. Statistically significant results from univariate analysis were included in multivariate analysis. Factors influencing CSF β-syn results were analyzed using multivariate linear regression, including WB CSF 14-3-3, Myoclonus and Akinetic Mutism

Abbreviations: *PWSC* periodic sharp wave complexes, *EEG* electroencephalogram, *MRI* magnetic resonance imaging, *CSF* cerebrospinal fluid, *β-syn* β-synuclein, *IQR* interquartile range, *N/A* not applicable

[a] Mann-Whitney *U* test

[b] Kruskal-Wallis H test

Evaluation of the diagnostic performance of CSF β-syn in various types of PrDs

The diagnostic accuracies of CSF β-syn for various types of PrDs were accessed by calculating the area under the ROC curve against total non-PrD cases, AD + PD cases, and VE + AE cases, separately. CSF β-syn in T188K- (AUC = 0.7334) and E200K-gCJD (AUC = 0.7297) had better diagnostic performance, followed by P102L-GSS (AUC = 0.6336), sCJD (AUC = 0.6116) and D178N-FFI (0.5506) (Fig. 2A). In terms of distinguishing AD + PD, all types of PrDs revealed satisfied performance, with higher AUC values for sCJD (0.7640), T188K-gCJD (0.8489), E200K-gCJD (0.8548), P102L-GSS (0.7689) and D178N-FFI (0.7210), respectively (Fig. 2B). In distinguishing VE + AE, all types of PrDs showed relatively lower AUC values (Fig. 2C).

To determine the potential cut-off values of CSF β-syn, the Youden index was calculated, which represents the best balance between sensitivity and specificity of the detection. The point corresponding to the highest Youden index represents the optimal cut-off. The values of the highest Youden index in the various groups of PrDs vs AD + PD were apparently higher than that of PrDs vs VE + AE (Table 3). Given the fatal nature of PrDs, high specificity of the diagnostic biomarkers should be preferentially considered. As shown in Supplemental Table 3, in the context of PrDs vs AD + PD at the point of approximate 70% specificity, the CSF β-syn cut-off was measured from 332.5 to 464.5 pg/ml among various PrDs, in which the sensitivities of T188K- and E200K-gCJD reached over 90%, those of sCJD and P102L-GSS were higher than 85% and that of D178N-FFI was 63.64%. At the point of about 80% specificity, the CSF β-syn cut-off raised from 796.5 to 948.5 pg/ml, while the sensitivities of T188K-gCJD, E200K-gCJD, P102L-GSS and sCJD still maintained at relatively high level (87.93, 84.38, 78.57 and 69.77%, respectively) but that of D178N-FFI dropped to 45.45%. In the context of PrD vs VE + AE at 70% specificity, the sensitivities of T188K- and E200K-gCJD decreased to 60 and 70% with a higher threshold (> 2500 pg/ml), while those of sCJD, P102L-GSS and D178N-FFI were below 40%. At the point of 80% specificity, the sensitivities all types PrDs decreased remarkably to less than 30%.

Discussion

The diagnosis of PrD in the last decades benefits from the highly sensitive and specific technique (i.e., the RT-QuIC) with assays of surrogate proteins reflecting neurodegeneration. However, the limitations of RT-QuIC application have prompted an ongoing pursuit of appropriate biomarkers [14]. As a candidate biomarker for synaptic degeneration and neuronal damage in PrDs and AD using digital ELISA [21] or targeted mass spectrometry [23], higher levels of β-syn in CSF and blood has been reported in the patients of CJD [24]. Given the current emergence of synaptic markers, further data is required

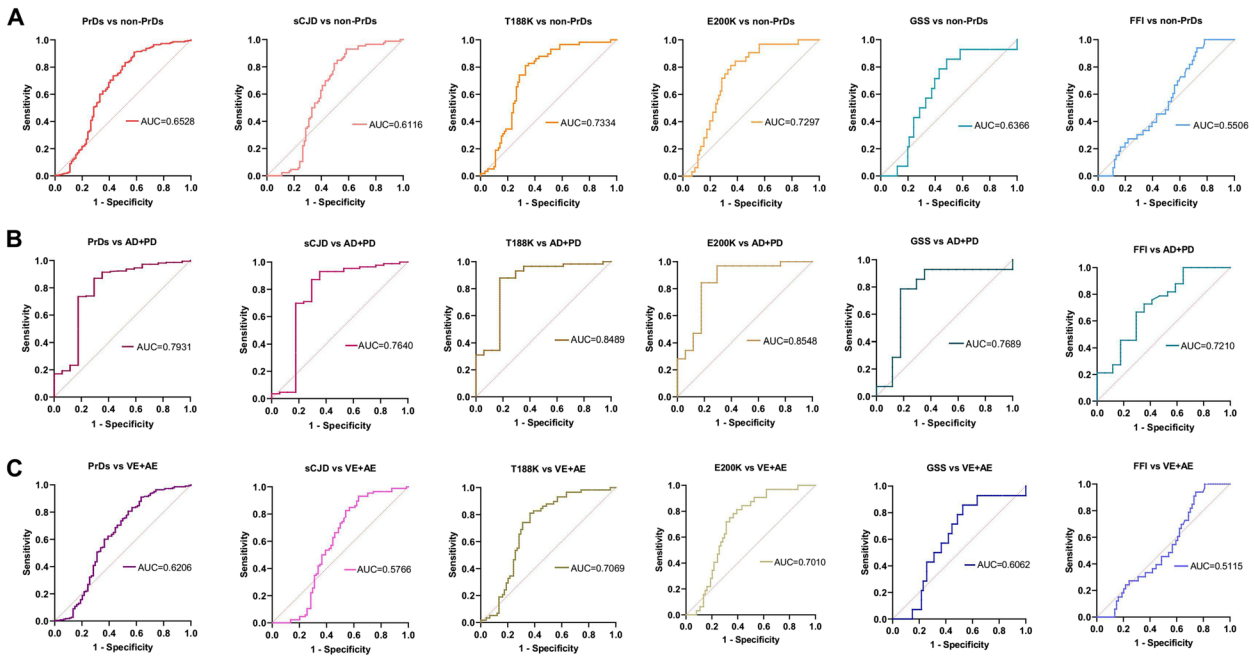

Fig. 2 CSF β-syn ROC Analyses in the Discrimination of PrDs and non-PrDs. ROC evaluations for the diagnostic performance of CSF β-syn in the discrimination of various types of PrDs with the groups of total non-PrDs (**A**), AD + PD (**B**) and VE + AE (**C**)

Table 3 Youden index cutoffs for the discrimination of prion disease from the other cohorts and the corresponding AUC, sensitivity, specificity, and positive likelihood ratio

Comparison		Youden index	AUC	Positive likelihood ratio	β-syn cut-off, pg/ml	Sensitivity (95% CI), %	Specificity (95% CI), %
AD + PD vs	sCJD	0.578	0.764	2.965	337.0	87.21 (78.53–92.71)	70.59 (46.87–86.72)
	T188K-gCJD	0.703	0.849	4.983	842.5	87.93 (77.12–94.03)	82.35 (58.97–93.81)
	E200K-gCJD	0.675	0.855	3.294	332.5	96.88 (84.26–99.84)	70.59 (46.87–86.72)
	P102L-GSS	0.609	0.769	4.452	809.5	78.59 (52.41–92.43)	82.35 (58.97–93.81)
	D178N-FFI	0.374	0.721	2.061	266.0	72.73 (55.78–84.93)	64.71 (41.30–82.69)
VE + AE vs	sCJD	0.295	0.577	1.465	271.5	93.02 (85.60–96.76)	36.49 (26.44–47.87)
	T188K-gCJD	0.445	0.707	2.221	1304.0	81.03 (69.15–89.07)	63.51 (52.13–73.56)
	E200K-gCJD	0.421	0.701	2.073	1159.0	81.25 (64.69–91.11)	60.81 (49.42–71.14)
	P102L-GSS	0.330	0.606	1.626	598.0	85.71 (60.06–97.46)	47.03 (36.34–58.52)
	D178N-FFI	0.196	0.512	1.264	145.5	93.94 (80.39–98.92)	25.68 (17.10–36.65)

Abbreviation: *AUC* area under the curve

to assess their potential clinical value. The objective of this study is to explore the potential screening and diagnostic value of CSF β-syn in the Chinese patients with PrDs and to understand the possible factors associated with CSF β-syn levels.

Here, we have identified significant elevation of CSF β-syn in Chinese patients of PrDs after onset, utilizing a commercial kit for β-syn on the Ella microfluidic system that offers a higher level of precision and a superior workflow with results available in less than 90 min. This finding is aligned with a previous study that CSF β-syn increased markedly in Caucasian sCJD patients [20]. We have also found, probably for the first time, the different elevating degrees of CSF β-syn among different genetic PrDs. The increased CSF β-syn levels in the patients of sCJD and gCJD seem to be associated with CSF 14-3-3 positive and the appearance of mutism, but not with disease duration. These findings support the potential utility of CSF β-syn as a biomarker for PrDs screening and diagnosis.

The CSF β-syn levels display different reactive profiles among the different types of PrDs, showing highest level in the group of gCJD (T188K and E200K), then, in the groups of sCJD and GSS (P102L), whilst no significant change in the group of FFI (D178N). It is postulated that the massive synaptic degeneration might lead to β-syn release from the presynaptic terminals into the extracellular, subsequently entering the CSF [20, 21, 24]. Thereby, the elevated β-syn level in CSF, possibly also in peripheral blood, may reflect neuronal damage. T188K-gCJD and E200K-gCJD show the similarity as sCJD in clinical, neuropathology and many other clinical and laboratory examinations such as EEG, MRI, CSF 14-3-3, CSF total tau, RT-QuIC, etc. [25, 26]. Compared to sCJD that possesses a wider range of differences in phenotypes likely due to the complicated PrP^{Sc} conformational structures and/or prion strains, the phenotypes of the patients of T188K-gCJD or E200K-gCJD are more identical. Higher and less wide range of CSF β-syn levels in those two types of gCJD cases may reflect the consistencies in PrP^{Sc} structures and phenotypes, including neuron damage. The clinical and neuropathology of GSS are different from sCJD, e.g., longer clinical duration, relatively lower levels of CSF 14-3-3 and total tau [27, 28]. Those features in GSS, at least in P102L-GSS, may represent a relatively slow process of neuron injury leading to relatively markedly lower CSF β-syn levels than gCJD. D178N-FFI is another very different type of PrDs, neuropathologically much less PrP^{Sc} deposit in the brain tissues. The positive ratios of CSF 14-3-3, CaM and the level of total tau are also remarkably lower than sCJD and many subtypes of gCJD [27, 29, 30]. Distinct change patterns of CSF β-syn in various types of PrDs may closely associate with the different PrP^{Sc} structures and neurotheologies.

We have also evidenced a good distinguishing performance of CSF β-syn between PrDs and the other two neurodegenerative diseases, AD and PD, but no significance in the discrimination with VE and AE. AD and PD also consist of different subtypes with different clinical and neuropathological characteristics. The disease progressions of AD and PD usually much slower than that of PrDs, which may also reflect a slow neuronal injury or damage. Additionally, the CSF specimens of most AD and PD cases in this study are sampled shortly after onset, who were referred as suspected CJD cases and later diagnosed as AD or PD clinically. The neuron damage and loss of AD and PD cases at early clinical stage possibly are less severe

than the patients of gCJD and sCJD in the clinical stage, which may partially explain the same observation in previous studies [20, 23, 24]. Contrary to AD and PD, the patients diagnosed as VE and AE seem to have similar increased levels of CSF β-syn in the current study. Adult VE or AE may display some neurological symptoms that are hard to be distinguished from PrDs clinically. Neuronal injury in VE (i.e., herpesvirus encephalitis) and AE usually occurs relatively quick, but the involved brain range is wide. Possibly, the relatively fast and wide neuronal damage is more closely related with the increase of CSF β-syn.

Besides of CSF 14-3-3 positive that is one of the biomarkers for neuron damage, our univariate and multivariate analyses of CSF β-syn levels in sCJD and gCJD patients have indicated statistical relationship with the appearance of mutism. As one of the clinical manifestations in the diagnostic criteria for CJD, akinetic mutism usually appear at the late stage of PrDs, which is characterized by extensive neuronal loss [31]. However, we do not observe significant association of CSF β-syn levels with the disease durations, although the median of CSF β-syn in the cases of sCJD and gCJD with relatively long duration (> 5 months) is relatively higher than those with short duration. Our previous study also did not find significant relationship of clinical duration with CSF 14-3-3 positive and other clinical and social factors [28]. Like CSF 14-3-3 positive and elevated total tau, the increased CSF β-syn in gCJD and sCJD can be also as a marker for rapid and extensive neuron damage, but not as a prognostic index for survival.

ROC analyses for the specificity and sensitivity of CSF β-syn in this study show an acceptable good performance in distinguishing PrDs from AD and PD, but much less efficient from VE and AE. Under our experimental condition here, we propose that the cut-off value of CSF β-syn is set as around 1000 pg/ml, which will reach to above 80% of specificity and about 70% (sCJD and GSS) to 85% (gCJD) sensitivity controlled with AD and PD cases. Our findings here suggest that measurement of CSF β-syn is significant for sCJD and certain types of genetic PrDs, at least providing an optional method for screening suspected PrDs premortem.

There are several limitations in this study. The sample size of the groups of AD and PD are small and the sampling times of AD and PD patients are mostly at the period shortly after disease onset. The sCJD cases are probable sCJD with a positive RT-QuIC, lacking neuropathological confirmation. The influence of pathological subtypes of PrP^{Sc} in the reactivity of CSF β-syn remains unknown.

Conclusion

Our findings indicate that CSF β-syn is likely a potential biomarker for distinguishing PrDs (gCJD, sCJD and GSS) from AD and PD, but is much less efficient from VE and AE. Further larger sample sizes are still needed to validate the feasibility for clinical usage.

Abbreviations

AD	Alzheimer's disease
AUC	Area under the curve
AE	Autoimmune encephalitis
β-syn	β-synuclein
CSF	Cerebrospinal fluid
CNS	Central nervous system
CNS-CJD	China National Surveillance for CJD
EEG	Electroencephalogram
FFI	Fatal familial insomnia
gCJD	Genetic CJD
GSS	Gerstmann-Straussler-Scheinker syndrome
IQR	Interquartile range
LLOQ	Lower Limit of Quantitation
MRI	Magnetic resonance imaging
MS	Mass spectrometry
N/A	Not applicable
PLR	Positive likelihood ratio
PrDs	Prion diseases
PD	Parkinson's disease
PWSC	Periodic sharp wave complexes
RT-QuIC	Real-time Quaking Induced Conversion
sCJD	Sporadic Creutzfeldt-Jacob disease
VE	Viral encephalitis

Supplementary Information

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Supplementary Material 1: Table 1, Table 2 and Table 3.

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Authors' contributions

Study design: BX, KX, XXJ, RDC, QS and XPD. Methodology: BX, KX, DLL, RHA, WWZ and CJL. Software: XXJ, RDC, LPG and CC. Formal analysis: BX, KX, DLL, RHA, QS and XPD. Writing original draft preparation: BX, KX, QS and XPD. Reviewing and editing: BX, KX, XXJ, RDC, DLL, RHA, WWZ, CJL, LPG, CC, QS and XPD. All authors interpreted the data and have drafted or substantively revised the work. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Committee of the National Institute for Viral Disease Control and Prevention, China CDC, under protocol 2009ZX10004-101. All participants provided informed consent by the Declaration of Helsinki and local clinical research regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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