Cerebrospinal Fluid Levels of Prodynorphin-Derived Peptides Are Decreased in Huntington's Disease

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ABSTRACT: Background: Huntington's disease (HD) is a devastating neurodegenerative disorder characterized by a selective loss of striatal medium spiny projection neurons (MSNs). Prodynorphin (PDYN) is enriched in a subpopulation of striatal MSNs. Postmortem brains of HD patients and rodent models have been demonstrated to have reduced levels of PDYN transcripts and the neuropeptide dynorphin.

Methods: Given the unmet need for novel pharmacodynamic HD biomarkers in the context of experimental huntingtin (htt)-lowering therapies, we investigated the levels of PDYN-derived peptides and neurofilament light (NfL) chain in the cerebrospinal fluid (CSF) from HD patients (n = 16), matched controls (n = 55), and patients with other neurodegenerative disorders (n = 70).

Results: PDYN-derived peptide levels were found to be substantially decreased in HD patients ($P < 0.0001$ in comparison to controls), whereas the NfL levels were elevated in all neurodegenerative disorders.

Conclusions: Our study suggests decreased PDYN-derived peptide levels in the CSF as a more specific biomarker for HD in comparison to NfL. © 2020 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: neurofilaments; prodynorphin; biomarker; Huntington's disease; frontotemporal dementia

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Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder caused by an expanded CAG trinucleotide repeat in the huntingtin (*HTT*) gene.[1] A hallmark of HD is progressive brain atrophy, particularly in the striatum.[2,3] These macroscopic alterations reflect, in part, a selective loss of striatal medium spiny projection neurons (MSNs).[4] There is a body of evidence highlighting the particular vulnerability of striatal MSNs.[4-6] MSNs are inhibitory, GABA-ergic neurons, distinguished by the expression of peptide co-transmitters acting on opioid receptors. Three genes coding for endogenous ligands acting on opioid receptors are known: prodynorphin (PDYN) or pre-pro-enkephalin B,[7] pro-enkephalin,[8] and pro-opiomelanocortin.[9] The expression of these genes is highly enriched in the striatum and hypothalamus in comparison to other regions in the brain.[10,11] The precursor protein PDYN is processed to the secreted peptides α/β-neoendorphin, dynorphin-A, dynorphin-B, leu-enkephalin, rimorphin, and leumorphin.[12] PDYN mRNA expression has been found to be downregulated in postmortem HD brains,[13] and striatal expression of PDYN has been found to be decreased in transgenic models of HD.[14,15] Decreased levels of dynorphin-A_{1-8} have been found in basal ganglia of HD patients postmortem.[16] The previously reported decrease in PDYN transcripts and PDYN-derived peptides in HD brains likely reflects the combined effects of transcriptional dysregulation and the loss of MSNs expressing the *PDYN* gene.

Neurofilaments are intermediate filaments of neurons, and they provide structural support for the axon caliber. Neurofilaments are heteropolymers composed of neurofilament light (NfL), neurofilament medium, and neurofilament heavy chains and α-internexin subunits.[17] NfL chain represents a biomarker of neurodegeneration,

elevated serum/CSF NfL levels being found in different neurodegenerative diseases, including HD.[18,19]

There is an unmet need to develop HD biomarkers to accelerate the development of new therapies. CSF represents an accessible body fluid to explore potential biomarkers, reflecting alterations in the brain.[20]

Based on robust evidence of a downregulation of PDYN in HD brains, we therefore set out to quantify PDYN-derived peptides reflecting PDYN expression in the CSF of HD patients, age-matched controls, and patients with other neurodegenerative disorders, to evaluate the utility of PDYN-derived peptides as a potential biomarker for HD, and to assess its specificity for HD in comparison to NfL.

Patients and Methods

Patients and CSF Collection

The local ethics committee of Ulm University approved the study (numbers 259/09 and 20/10), and the participants provided written consent. The study comprised 141 patients: 16 HD patients, 55 matched non-neurodegeneration controls, and 70 participants with other neurodegenerative diseases. The latter consisted of 17 sporadic amyotrophic lateral sclerosis (sALS), 26 Alzheimer's disease/minimal cognitive impairment (AD/MCI), 16 frontotemporal dementia (FTD), and 11 Parkinson's disease (PD) patients. The patients were diagnosed according to the current diagnostic criteria.[21-25] The abnormalities prompting admission of the matched non-neurodegenerative controls are listed in Supplementary Table S1.

Measurements of NfL Chain and Prodynorphin

CSF was collected by lumbar puncture, centrifuged, and stored at −80°C. NfL levels were measured on an HD-1 Analyzer (Simoa) using NF-light advantage kits that employ an immunoassay (Quanterix, Lexington, MA) according to the manufacturer's instructions. PDYN was measured using targeted liquid chromatography mass spectrometry. The detailed description of this method is provided in the Supplementary Data.

Results

The demographic characteristics of the study groups are summarized in Table 1. The cohort of the HD patients did not differ in age from the non-neurodegenerative controls ($P > 0.999$). The HD cohort was younger than the cohort of patients with other neurodegenerative diseases, as expected; within this group, participants with distinct conditions were of comparable age ($P > 0.999$). The clinical scores of HD participants are provided in Supplementary Table S2.

To robustly assess the expression of PDYN at the protein level, we established a targeted liquid chromatography mass spectrometry method for the quantitation of two PDYN-derived peptides in the CSF. The sequence of the measured PDYN-derived peptides A and B and examples of peptide peaks are provided. The assessed peptides lay within the pro-peptide 21–172 region and did not contain the bioactive DYN molecules (eg, DYNA1-17 and DYNB1-13) (Supplementary Figure S1). For both peptides, the established method showed an intra-assay variation (<13.5%) and a similar inter-assay variation (<13%). We observed a strong correlation between the levels of the two PDYN-derived peptides ($r = 0.75$, $P < 0.0001$), suggesting a consistent and reliable estimate of PDYN levels in the CSF.

The PDYN-derived peptides A and B showed similar results, so we used the mean of the two peptides in the comparisons. The detailed results of the individual peptides are provided (Supplementary Figure S2).

As shown in Figure 1A, the levels of PDYN-derived peptides in the CSF of HD patients were markedly decreased in comparison to the age-matched non-neurodegenerative controls ($P < 0.0001$). The levels of PDYN-derived peptides in the CSF of HD patients were

TABLE 1. Demographic characteristics and biomarker levels in the studied groups

	CON	HD	sALS	AD/MCI	FTD	PD
Cohort size	55	16	17	26	16	11
Gender (% men)	60.0	68.8	76.5	30.8	43.8	90.9
Age in years[a] (range)	54.5 (45.4–64)	51.0 (41–56.1)	63.9 (58.8–68.1)	68.5 (66–72)	63.9 (55.3–69.4)	70.0 (55–74)
CSF NfL[b] in pg/mL (range)	772 (543–1151)	3165 (2435–4691)	4967 (2811–10,368)	1730 (1569–1963)	3626 (1341–6245)	1164 (710–2114)
CSF PDYN[c] in AU (range)	0.48 (0.38–0.56)	0.25 (0.16–0.31)	0.47 (0.36–0.63)	0.48 (0.36–0.56)	0.39 (0.32–0.45)	0.31 (0.26–0.64)

Given values are the median and interquartile range. The Kruskal–Wallis test and Dunn's multiple-comparison test were applied, and significance values are as follows: [a]HD versus AD/MCI $P < 0.0001$, versus PD $P = 0.0012$, versus FTD $P = 0.0341$, and versus sALS $P = 0.0139$. AD/MCI versus CON $P < 0.0001$ and PD versus CON $P = 0.0123$. [b]HD versus CON $P < 0.0001$, versus sALS $P < 0.0001$, versus AD/MCI $P < 0.0001$, versus FTD $P < 0.0001$, and versus PD $P = 0.0007$. HD versus PD $P = 0.0238$, sALS versus PD $P = 0.0069$. [c]HD versus CON $P < 0.0001$, versus sALS $P = 0.0023$, and versus AD/MCI $P = 0.0012$.

CON, controls; HD, Huntington's disease; AD/MCI, Alzheimer's disease/minimal cognitive impairment; sALS, sporadic amyotrophic lateral sclerosis; FTD, frontotemporal dementia; PD, Parkinson's disease; NfL, neurofilament light chain; PDYN, prodynorphin-derived peptides; AU, arbitrary unit defined as the mean of the measured ratio light to heavy of the measured PDYN-derived peptides.

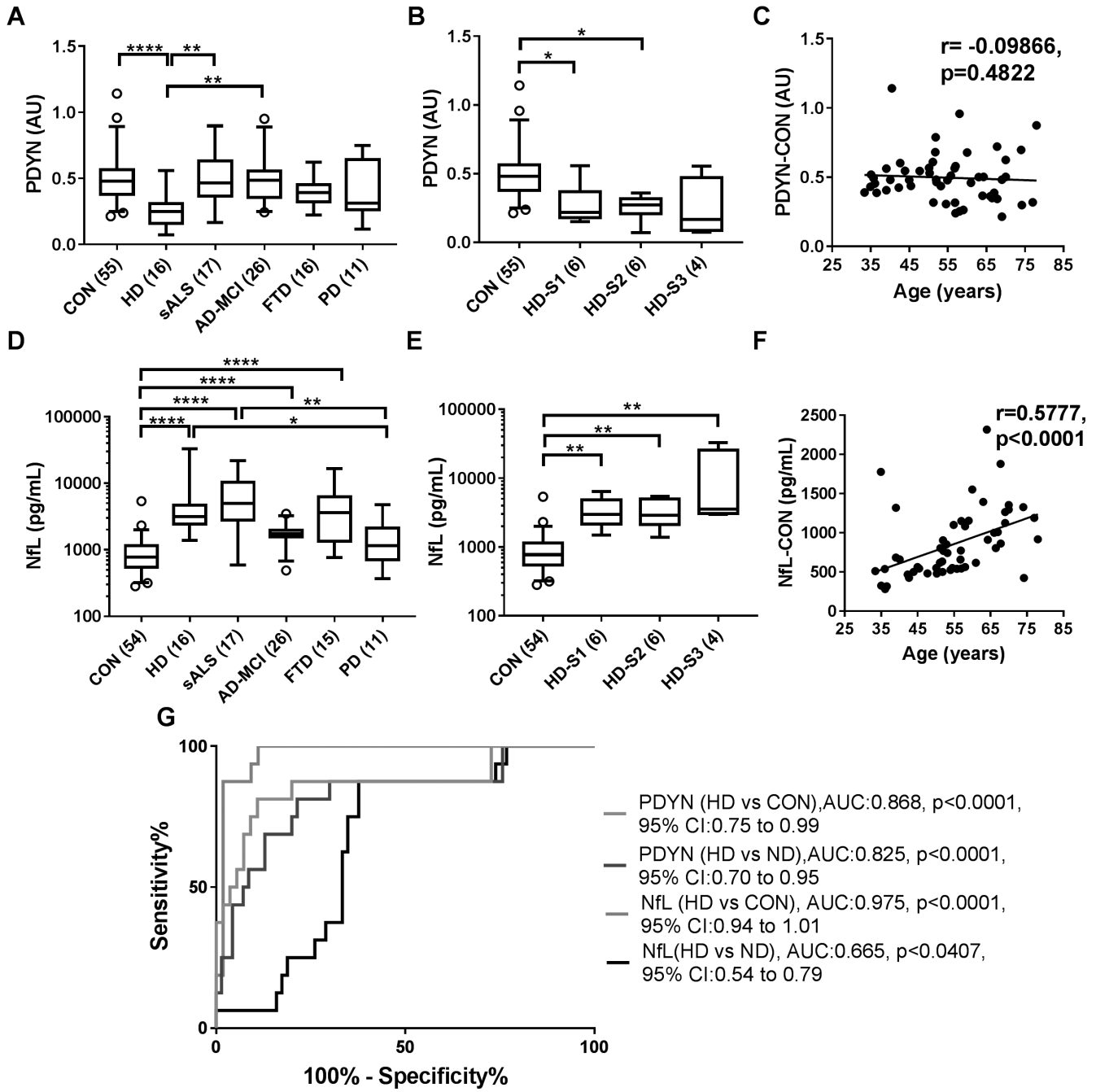

FIG. 1. Levels of prodynorphin (PDYN)-derived peptides in the cerebrospinal fluid (CSF) are downregulated, and levels of neurofilament light (NfL) are upregulated in Huntington's disease (HD) patients. However, only PDYN-derived peptides differentiate HD from other neurodegenerative diseases. (**A**) CSF PDYN–derived peptide levels in the different cohorts. (**B**) CSF PDYN–derived peptide levels as a function of disease stage (measured by total functional capacity [TFC] scores). (**C**) Correlation between age at sampling and the CSF PDYN–derived peptide levels in the control cohort. (**D**) CSF NfL levels in the different cohorts. (**E**) CSF NfL levels as a function of clinical severity of HD expressed as clinical stages (measured by TFC scores). NfL levels are plotted on a 10-logarithmic scale. Boxes are the median levels and interquartile range; whiskers are 5% and 95% percentiles. Points represent values below 5% percentile and above 95% percentile. Asterisks refer to statistically significant differences with unpaired Kruskal–Wallis test and Dunn's post hoc test, $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$. (**F**) Correlation between age at sampling and CSF NFL levels for the cohort of controls. (**G**) Receiver operating characteristic (ROC) curves of CSF NfL and CSF PDYN for differentiation between HD patients and controls (NfL in blue, PDYN in orange) as well as HD and other neurodegenerative conditions as a conglomerated group (NfL in black, PDYN in purple). CON, controls; HD, Huntington's disease; AD/MCI, Alzheimer's disease/minimal cognitive impairment; sALS, sporadic amyotrophic lateral scleroses; FTD, frontotemporal dementia; PD, Parkinson's disease; and ND, neurodegenerative conditions as a conglomerated group (sALS, AS-MCI, FTD, and PD). CI, confidence interval; AUC, area under the curve. PDYN (AU) is the mean of the light-to-heavy ratios of the two peptides measured. The ratio L/H of a peptide is calculated by dividing the AUC of the native CSF peptide over the AUC of the spiked heavy standard peptide with a known concentration. The PDYN-derived peptides were measured using targeted liquid chromatography mass spectrometry. [Color figure can be viewed at wileyonlinelibrary.com]

also decreased in comparison to sALS patients ($P = 0.0023$) and AD/MCI patients ($P = 0.0012$). The levels of PDYN-derived peptides in the CSF of sALS, AD/MCI, and FTD patients were similar to those in the non-neurodegenerative controls. The levels of PDYN-derived peptides in the CSF of PD patients showed a declining trend in comparison to non-neurodegenerative controls. Pairwise comparisons of sALS, AD/MCI, FTD, and PD did not show significant differences in PDYN levels measured (Fig. 1A). In addition, we observed a trend toward decreasing PDYN-derived peptide levels as a function of the clinical severity of HD, as measured by the total functional capacity (TFC) score (Fig. 1B). PDYN-derived peptides did not correlate with age in the control group ($r = 0.099$, $P = 0.48$) (Fig. 1C). PDYN-derived peptides did not correlate with age in the HD group ($r = 0.17$, $P = 0.53$).

NfL levels in the CSF were significantly increased in HD, sALS, FTD, and AD/MCI patients when compared to controls ($P < 0.0001$ for all comparisons) (Fig. 1D) but not in PD. In comparison to PD, NfL levels were elevated in sALS ($P = 0.007$) (Fig. 1D) and in HD ($P = 0.024$) (Fig. 1D). All HD patients displayed increased NfL CSF levels in comparison to controls, with no obvious increase as a function of clinical severity, measured by TFC scores (Fig. 1E). In the control group, the levels of NfL in the CSF strongly correlated with age ($r = 0.578$, $P < 0.0001$) (Fig. 1F), whereas there was no correlation between the levels of NfL and age in HD patients ($r = -0.181$, $P = 0.499$). In the cohort of HD patients, the levels of NfL showed a robust correlation with disease burden score ($r = 0.539$, $P = 0.033$). No correlation between the levels of NfL and those of PDYN-derived peptides was observed.

To evaluate the efficacy of the PDYN and NfL measurements in distinguishing HD from non-HD, we ran a receiver operating characteristic (ROC) analysis of PDYN-derived peptide levels in the CSF of HD patients compared to controls (HD vs. CON). The analysis showed an area under the curve (AUC) of 0.87 and a specificity and sensitivity of 89.1% and 81.25%, respectively (cutoff level <0.31 AU, Fig. 1G-orange line). ROC analysis of the levels of PDYN-derived peptides of HD patients compared to all other patients suffering from neurodegenerative conditions combined (HD vs. neurodegenerative) yielded an AUC of 0.825 and a specificity and sensitivity of 81.3% and 78.6%, respectively (cutoff <0.31 AU, Fig. 1G-violet line). The ROC analysis of CSF NfL of HD patients and controls demonstrated an AUC of 0.975 and a specificity and sensitivity of 100% and 88.9%, respectively (cutoff >1372 pg/mL, Fig. 1G-blue line). Comparing HD patients with patients suffering from other neurodegenerative conditions, the AUC was 0.665, and the specificity and sensitivity were 87.5% and 62.32%, respectively (cutoff >2291 pg/mL, Fig. 1G-black line). ROC analysis of PDYN for each disease group compared to controls is provided in Supplementary Figure S3.

Discussion

This is the first report demonstrating the decreased levels of PDYN-derived peptides in the CSF of HD patients in comparison to controls. This decrease may be a unique characteristic of HD, as a comparable decrease was not observed in the other neurodegenerative disorders studied.

The decrease in CSF PDYN levels observed in HD patients is consistent with previous reports demonstrating decreased parenchymal levels of dynorphin-$A_{1\text{-}8}$ in HD brains.[13,16]

The enkephalinergic neurons of the indirect striatal output pathway appear to be particularly vulnerable in HD patients, and they may rapidly degenerate in presymptomatic HD patients.[26] Previous studies have shown decreased CSF proenkephalin levels and no change in CSF levels of substance P in HD patients.[27,28] Presumably, the rapid degeneration of enkephalinergic neurons and the widespread distribution of substance P neurons in other brain regions limit the utility of CSF substance P and proenkephalin as a progression marker in HD patients. In contrast, the degeneration of dynorphin neurons, which are mainly in the direct striatal output pathway, is relatively mild early and continues more steadily with the progression of the disease,[26] which emphasizes the potential use of CSF PDYN as a progression marker.

Because the loss of dopamine can decrease the PDYN expression from direct striatal pathway projection neurons,[29] a decrease in the levels of PDYN in the CSF from PD patients is expected. The observed decrease in CSF PDYN levels may not have reached statistical significance in our study because of a possible variation in dopamine levels in the PD patients studied. It is also possible that levodopa therapy in at least some PD patients somewhat normalized PDYN expression, because dopamine agonists are known to increase PDYN expression in animal models.[29] It is worth mentioning that the decrease in CSF PDYN levels is expected to be more pronounced in HD in comparison to PD patients, as, unlike in HD patients, the direct pathway projection neurons of the striatum are not lost in PD patients.

The PDYN-derived peptides assessed in our assay were specific to PDYN and did not include the sequence of leucine-enkephalin (Tyr-Gly-Gly-Phe-Leu), which is also found in DYNA1-17 and DYNB1-13, so there was no risk of detecting other peptides (ie, leucine-enkephalin) and mistaking them for a PDYN peptide. An immunoassay measuring these peptides or other peptides of PDYN[30] with specific antibodies might be serviceable for assessing the levels of PDYN in the CSF of HD patients.

In line with a recently published meta-analysis,[31] our data highlight that NfL is elevated in a broad range of neurodegenerative disorders, indicating the limited use of CSF NfL levels in differentiating between distinct neurodegenerative disorders (with the exception of ALS). In comparison to NfL, the decrease in CSF PDYN in HD patients appears to be a more specific/selective biomarker. However, the range of neurodegenerative conditions studied here was limited and did not include some conditions with marked striatal atrophy like multiple system atrophy and the primary progressive aphasia subtypes of FTD. Another obvious limitation of our study is the small sample size.

In conclusion, the levels of PDYN-derived peptides in the CSF are promising biomarker candidates in the context of HD patients. Further studies are needed to establish and validate these measurements as a progression marker and potentially as a response marker.

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Supporting Data

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

The Spectrum of Repetitive Behaviors Associated with Subacute Sclerosing Panencephalitis

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ABSTRACT: Background: Repetitive behaviors refer to a broad class of responses ranging from stereotypic body movements to impulsive/compulsive behaviors. They may be associated with neurological disorders.

Methods: This is a case series of six subacute sclerosing panencephalitis (SSPE) patients who presented with a wide spectrum of repetitive motor behaviors and vocalizations.

Results: Repetitive motor behaviors involved the upper limbs in all patients and lower limbs in 3 patients. The repetitive movements in the upper limbs were clapping, finger-clicking, hand rubbing, flailing, and dystonic posturing. In the lower limbs, the repetitive movements were rubbing with the heel, pelvic thrusting with flexion extension of the leg, and foot tapping. The spectrum of vocalizations included palilalia, whistling, grunting with spitting, and pathological crying. Repetitive behaviors were the presenting features in 2 patients.

Conclusions: This case series expands the spectrum of repetitive behaviors seen in neurological disorders associated with brain infections.

Key Words: stereotypy; vocalization; magnetic resonance imaging; electroencephalography; measles IgG antibodies

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Subacute sclerosing panencephalitis (SSPE) is a slowly progressive brain disorder that affects younger age groups and is caused by a mutant measles virus.[1] Higher-income countries have seen a significant decline in SSPE cases, whereas the incidence is still high in India and the Middle Eastern countries.[2] Clinically, SSPE is characterized by a progressive course, and death occurs in the majority of patients.[3,4] Some patients may have acute-fulminant SSPE characterized by a rapid disease course culminating in death within 6 months.[1]A history of measles or vaccination is present in the majority of patients; however, some patients have been reported without such a history.[2,5] Movement disorders can be one of the characteristic symptoms of SSPE patients, but repetitive behaviors have seldom been reported.[6-8] Here we highlight a series of 6 patients where repetitive behaviors were the predominant clinical features.

Methods

This study is a case series of 6 patients with SSPE in whom repetitive behaviors were observed in our movement disorders outpatient clinics at Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, University of Delhi, India. Their detailed demographic, clinical, and lab data (Table 1) were recorded, and repetitive behaviors were videotaped (see Video S1) after informed consent was obtained from the parents. Repetitive behaviors were classified as repetitive motor behaviors (including stereotyped motor movements) and vocalizations.

All patients underwent hematological and urine investigations, including autoimmune workup, magnetic resonance imaging (MRI) (Fig. 1A–R), cerebrospinal fluid (CSF) study, and electroencephalography (EEG). Measles IgG antibodies were measured in the CSF and serum using enzyme immunoassays. Anti-*N*-methyl-D-aspartate receptor (NMDAR) antibodies in the CSF and serum were measured using indirect immunofluorescence assay on transfected cell lines in all patients. A diagnosis of SSPE was made using the Dyken criteria (clinical, EEG, and elevated levels of IgG measles antibodies in CSF).[9]