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Detection of Parkinson disease- associated α-Synuclein in cerebrospinal fluid: a feasibility study

» INTRODUCTION

Neurodegenerative disorders are characterized by the formation and deposition of abnormal isoforms of physiologically occurring proteins primarily in the brain of affected individuals. In the case of Parkinson's disease (PD) and dementia with Lewy-bodies (DLB), this is α-synuclein, a presynaptic protein, which is deposited under pathological conditions in neurons and neuronal processes.

» AIMS

The detection of pathologically relevant formations of α-synuclein can provide additional manifestations for accompanying diagnostics. Due to the frequent co-occurrence of Lewy body pathology with Alzheimer's disease (AD) pathology, it is crucial to differentiate it from pure AD forms. It is therefore most desirable to make use of it not only in autopsies, but also for early clinical diagnosis, while the patient could still potentially benefit from the result.

» ANTIBODY α-SYNUCLEIN 5G4

α-Synuclein pathology is present as tiny dots, thin neurites, and larger amorphous depositis in the subependymal area, as well as tiny dots between ependymal cells in the brains of individuals with neuropathologically proven PD. The **Anti-human α-synuclein 5G4, monoclonal antibody** (Analytik Jena) shows high specificity for the disease-specific forms, including high molecular weight fraction of α-sheet rich oligomers, while no binding to primarily disordered oligomers or monomers is observed.

It was demonstrated, that diseases-associated α-synuclein (d-α-syn) deposits in the ependymal layer of the ventricles and aqueduct in PD and DLB cases are detected **(Figure 1)**, thus it is likely that it can be determined in CSF.

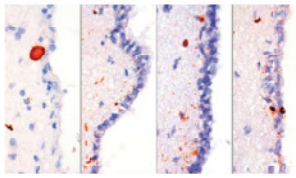

Figure 1 | Light microscopic immunostaining patterns of α-synuclein using antibody 5G4. Tiny dots, thin neurites in the subependymal area, as well as tiny dots between ependymal cells and amorphous plaques**.

» CASE SELECTION AND ELISA PERFORMANCE

A study of 22 cases of neuropathologically confirmed cases, among them patients with α-synucleinopathy with Lewy-related pathology (7 cases; 2 of them clinically classified as Parkinson's disease dementia (PDD) and 5 as DLB), with AD (6 cases), and 9 control patients was performed. Systematic neuropathological examination was carried out including immunohistochemistry for d-α-syn to confirm or rule out the presence of Lewy body pathology. Indeed, α-synuclein pathology was excluded in all cases with AD and controls without neurodegenerative disorders **(Table1)**.

ELISA plates coated with antibody 5G4 or 10D2 (Analytik Jena) were used for incubation with 100 µl of CSF diluted 1:1 in PBS-based dilution buffer. Controls and synthetic standards containing target antigens were incubated in parallel to patient samples for 24 h at 2 - 10°C. Plates were washed 5 times, and detection antibodies (Analytik Jena) were added for 90 min at room temperature. All plates were washed 10 times and antibody reactions were developed using ECL substrate FEMTO. For all statistical analyses comparing the diagnostic groups of patients M-W tests were applied.

Case	Sex	Age	Disease	Neuropathology
1*	m	78	DLB	LBP, Braak stage 4, BBVI, CAA, Argyrophilic grain disease.
2*	w	88	PDD	LBP, Braak stage 4, BBV, CAA, Limbic TDP-43 proteinopathy.
5	w	80	DLB	LBP, Braak stage 4, Progressive supranuclear palsy (tauopathy), Vascular encephalopathy, Limbic LUT-1 proteinopathy.
4*	m	73	DLB	LBP, Braak stage 5, BB4, Metabolic gliosis, Meningocele leukemica.
5	m	72	DLB	LBP, Braak stage 6, BBV, Vascular encephalopathy.
6	w	81	DLB	LBP, Braak stage 6, BBV, CAA.
7*	w	73	PDD	LBP, Braak stage 6, BBV, CAA.
8	w	85	AD	BBVI, Thalamic microinfarktion.
9	w	82	AD	BBVI, CAA.
10	w	88	AD	BBVI, CAA, Bilateral infarction occipital lobe, Cerebellar infarction
11	w	90	AD	BBVI, Severe atherosclerosis with infarcts in basal ganglia, occipital lobe and cerebellum.
12	w	84	AD	BBV, infarction right parietal lobe.
13	w	85	AD	BBVI, CAA.
14	m	48	C	Chronic meningoencephalitis (neurolues), Metabolic gliosis.
15	w	49	C	Binswanger encephalopathy.
16	w	78	C	Angiotropic B-cell Lymphoma.
17**	m	71	C	Viral polioencephalitis (TBE).
18	w	93	C	Multi-infarct encephalopathy.
19	m	59	C	Viral polioencephalitis (TBE).
20	w	68	C	Viral polioencephalitis, Acute ischemic/hypoxic encephalopathy.
21	m	49	C	Multi-infarct encephalopathy, Metabolic gliosis.
22	m	56	C	Limbic encephalitis.

Table 1 | Cases included in the study (DLB = dementia with Lewy bodies; PDD = Parkinson's disease dementia; LBP = Lewy body pathology; BB = Braak & Braak stageing of neurofibrillary degeneration (I – VI); AD = definite Alzheimer's disease; CAA = cerebral amyloid angiopathy; TBE = tick borne encephatitis

RESULTS

Total α-Synuclein (t-α-syn) in the CSF in cases with Lewy body pathology (LBP) and Alzheimer's diseases (AD), as well as a control group was determined using the described ELISA. The level of t-α-syn in LBP cases was dropped compared to AD patients and was significantly lower compared to control groups (p=0.016) **(Figure 2 A)**. By contrast levels of d-α-syn was - on average - higher in PDD/DLB than in other groups **(Figure 2 B)**. The ratio of t-α-syn to d-α-syn was calculated for each sample showing a significant difference only when comparing PDD/DLB with controls (p=0.002) **(Figure 2 C)**.

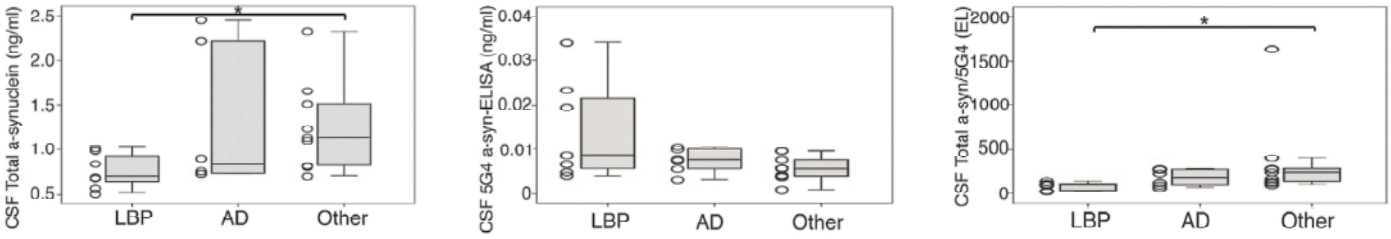

Figure 2 | Box-plot demonstration of levels of total (left) and disease-specific (middle) α-synuclein by ELISA in the CSF in cases with LBP and AD as well as in controls. Furthermore the results for the ratio of t-α-syn to d-α-syn in each sample are shown (right).

CONCLUSIONS

In this study evidence was provided that combined detection of t- α-syn and d-α-syn might be a promising tool for the in-vivo differentiation of patients with PDD/DLB from other types of dementia. Originating from this feasibility study Analytik Jenas **Human α-Synuclein MONO ELISA** and the corresponding **Human α-Synuclein PATHO ELISA** for detection of t-α-syn and d-α-syn, respectively, have been towards and will provide a sufficient differential diagnosis of AD from PDD/DLB. Currently the assays are further investigated (validation study using larger cohorts) to evaluate the exact specificity and sensitivity of a promising diagnostic for examination of α-Synucleinopathies independent of the sample input material (CSF, plasma or serum).

Reference:

* Unterberger U, Lachmann I, Voigtländer T, Pirker W, Berghoff AS, Flach K, Wagner U, Geneste A, Perret-Liaudet A, Kovacs GG (2014) Detection of disease-associated -synuclein in the cerebrospinal fluid: a feasibility study. Clin Neuropathol 33(5):329-34.

** Kovacs GG (2014) Clinical Neuropathology image 5-2014: α-synuclein pathology in the ependyma in Parkinson's disease

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