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# Cerebrospinal fluid and plasma clusterin levels in Parkinson's disease

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ABSTRACT

Clusterin is a multifunctional chaperone protein that has repeatedly been linked to Alzheimer's disease (AD) pathogenesis and, more recently, also to Parkinson's disease (PD) by both genetic and proteomic analyses. Although clusterin is detectable in cerebrospinal fluid (CSF) and plasma, studies comparing clusterin levels in PD patients and controls have been scarce and yielded conflicting data. The aim of the present study was to determine whether CSF and/or plasma clusterin levels differ between PD patients and controls and are related to disease severity. We measured CSF and plasma clusterin levels in a group of 52 PD patients and in 50 age-matched neurologically healthy controls and found that clusterin levels in CSF and plasma were not different between the two groups. Furthermore, clusterin levels in CSF and plasma were not associated with disease duration, stage or severity. CSF clusterin levels did, however, correlate with CSF levels of total tau, phospho-tau and amyloid-β-42. We elaborate on the identified correlations between levels of clusterin and AD related proteins and on possible explanations for the discrepant findings in clusterin studies in PD so far.



## 1. Introduction

Clusterin is a multifunctional glycoprotein that has been related to cholesterol and lipid metabolism, regulation of complement mediated cell lysis, as well as to inhibition of neuronal apoptosis [1]. Furthermore, clusterin acts as an extracellular chaperone that maintains stressed proteins in a soluble state, thereby preventing their precipitation [2].

A role for clusterin in Alzheimer's disease (AD) pathogenesis has become apparent from genome-wide association studies in which certain polymorphisms in CLU, the clusterin encoding gene, were found to confer susceptibility for AD [3,4]. In addition, expression studies showed up-regulated clusterin expression in AD-affected brain areas [5] and levels of clusterin were reported to be increased in cerebrospinal fluid (CSF) [6] and plasma [7] of AD patients. Moreover, higher plasma clusterin levels were associated with more severe cognitive dysfunction [7]. Furthermore, clusterin was found to co-localize with amyloid plaques and neurofibrillary tangles [8] in immunohistochemical studies.

Recently, a specific CLU single-nucleotide polymorphism (SNP; rs11136000) was reported to be associated with Parkinson's disease (PD) [9]. This association was most pronounced in PD patients with dementia and independent of APOE genotype and known risk factors for PD [9], suggesting that cortical Lewy Body pathology or concomitant AD pathology in the PD patients might play a role.

Using unbiased quantitative proteomic techniques, increased levels of clusterin were observed in both plasma [10] and CSF [11,12] of PD patients. These studies had a relatively small sample size. In two studies lumbar CSF clusterin levels were evaluated in larger numbers ($n = 11–32$) of PD patients and controls. The results of these studies were not in accord, clusterin levels being unchanged in one study [13] and increased in the other [14]. The increased clusterin levels reported were mainly found in patients with a short (≤2 years) disease duration [14]. Consequently, it remains unclear whether CSF or plasma clusterin levels differ between PD patients and controls, and whether clusterin could potentially serve as a diagnostic marker for PD.

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The aim of the present study was to evaluate whether CSF and/or plasma clusterin levels differ between PD patients and age-matched neurologically healthy controls. We compared plasma clusterin and lumbar CSF clusterin levels between 52 well-characterized PD patients and 50 controls, and analyzed the relationship between clusterin levels and disease duration, stage and severity of motor symptoms. In addition, we investigated whether clusterin levels were related to those of the AD-related proteins amyloid-β-42 (Aβ42), total tau (T-tau) and phospho-tau (P-tau).

## 2. Methods

### 2.1. Study population

For this study we included 52 PD patients from our outpatient clinic for movement disorders, as well as 50 self-declared healthy controls recruited through an advertisement in the periodical of the Dutch Parkinson Foundation. PD was diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank (UK-PDSBB) clinical diagnostic criteria [15]. Patients were included only if they were able to understand the study aim and procedures, and if no signs of dementia were detectable upon Mini-Mental State Examination (MMSE) and/or neuropsychological assessment. In the controls, dementia was excluded using the Cambridge Cognitive Examination (CAMCOG) scale [16]. Patients and controls underwent a standardized clinical assessment that included their medical history and a neurological examination. Severity of parkinsonism and disease stage in the "on" state were rated using the motor subscale of the Unified Parkinson's Disease Rating Scale (UPDRS-III) [17] and the Hoehn and Yahr classification [18], respectively. In addition, PD patients were divided into clinical subgroups according to Lewis et al. [19]: (1) younger onset, (2) tremor-dominant or (3) non-tremor dominant. None of the PD patients fulfilled the criteria for the rapid disease progression subtype. The study was approved by the local ethics committee of the VU University Medical Center and all subjects gave written informed consent.

### 2.2. Cerebrospinal fluid (CSF) and plasma samples

CSF was obtained by lumbar puncture, collected in polypropylene collection tubes, routinely assayed for cell counts, centrifuged at 1800× g at 4 °C for 10 min, aliquoted and stored at −80 °C within 2 h, in line with published guidelines [20]. Only samples containing less than 500 erythrocytes per microliter were included in the study, because traces of blood, in which clusterin expression is around 15 times higher than in CSF [21], may influence CSF clusterin levels. EDTA plasma was collected directly before or after lumbar puncture, centrifuged at 1800× g at 4 °C for 10 min, aliquoted and stored at −80 °C within 2 h.

### 2.3. Assays

Clusterin was quantified by an in-house sandwich ELISA. In short, disposable microtiter plates (Costar 9018, Corning) were coated with a clusterin-specific mouse monoclonal antibody (clone G7; [21]) and blocked with PBS containing 2% milk, where after 100 μL of either CSF samples (1:200 diluted) or EDTA plasma samples (1:5000 diluted) were added to the wells in duplicate and incubated for 1 h at room temperature. Bound clusterin was detected with biotinylated rabbit anti-human clusterin polyclonal antibody (Alexis Biochemicals, Enzo Life Sciences, Zandhoven, Belgium) and visualized after subsequent incubations with streptavidin poly-HRP (1:10,000 diluted; Sanquin, Amsterdam, The Netherlands), and 3,5,3′,5′-tetramethylbenzidine (TMB; Sigma, Germany). The reaction was stopped using sulfuric acid after 20 min and the absorbance was measured at 450 nm in a spectrophotometer (Bio-Tek Synergy HT, Winooski VT, USA). Clusterin purified from human plasma by affinity chromatography with Sepharose 4B coupled G7 monoclonal antibodies as described previously [22], was serially diluted to prepare a calibration curve. The linear range of the assay was from 0.5 to 38.9 ng/mL, the inter-assay and intra-assay coefficients of variation were 8.8% and 1.4%, respectively. CSF levels of Aβ42, T-tau and P-tau were determined using commercially available ELISAs (Innotest®; Innogenetics, Gent, Belgium) as described previously [23]. The technicians performing the ELISAs were not aware of the clinical diagnoses.

### 2.4. Statistical analysis

Statistical analysis was performed using Statistical Package of the Social Sciences software version 15.0 (SPSS, Chicago, IL, USA). T-tau and P-tau data were square root transformed, while plasma clusterin data required inverse transformation to obtain a normal distribution. Group comparisons between PD patients and controls were performed using univariate analyses of variance (ANOVA) for continuous data, corrected for age and gender, while group comparisons within the PD patients were corrected for age alone. Mann–Whitney *U* tests were used for ordinal data and chi-squared tests for categorical data. Correlations were assessed using bivariate Pearson and Spearman's rank correlation coefficients when appropriate. A Kruskal–Wallis test was used to compare CSF and plasma clusterin data between clinical subgroups of PD patients. For CSF clusterin, we excluded 1 outlier (PD patient) with a clusterin concentration higher than 10 ng/μl (>3 standard deviations from the mean). For plasma clusterin, we excluded 2 PD patients with a clusterin concentration >3 standard deviations from the mean. These exclusions did not alter the outcome of this study (data not shown).

Standard in-house diagnostic cut-offs were used for Aβ42 (>550 pg/mL), T-tau (<375 pg/mL) and P-tau (<52 pg/mL) [23] that were based on the optimal separation of AD patients from patients with subjective memory complaints. Statistical significance was set at $p < 0.05$.

## 3. Results

PD patients and controls were matched for age, but not for gender (Table 1). CSF clusterin levels did not differ between male and female subjects in patients or controls (PD $p = 0.82$; controls: $p = 0.88$). In PD patients, plasma clusterin levels were higher in females compared to males (female PD 79.3 ± 10.7 ng/μl; male PD 69.1 ± 9.4 ng/μl; $p = 0.003$). No difference was observed for controls ($p = 0.22$). In both PD patients and healthy controls, plasma and CSF clusterin levels did not correlate significantly with age, although in controls a trend towards higher CSF clusterin levels with increasing age was observed ($r = 0.25$, $p = 0.08$).

No significant differences in mean CSF or plasma clusterin levels were observed between PD patients (CSF clusterin mean ± SD: 4.9 ± 1.39 ng/μl; plasma clusterin: 73.2 ± 11.1 ng/μl) and controls (CSF clusterin: 4.6 ± 1.1 ng/μl; $p = 0.19$; plasma clusterin 76.1 ± 12.6 ng/μl; Fig. 1). Furthermore, we did not find correlations between CSF or plasma clusterin levels and disease duration, Hoehn and Yahr stage, or disease severity as measured by the UPDRS-III. Also, no significant differences in CSF or plasma clusterin levels were found between patients with a disease duration shorter than 3 years and patients with a longer disease duration (CSF clusterin: $p = 0.31$; plasma clusterin: $p = 0.45$). CSF and plasma clusterin levels did not differ between distinct clinical PD subgroups (CSF clusterin: younger onset 5.4 ± 1.3 ng/μl; tremor-dominant 4.9 ± 2.0 ng/μl; non-tremor dominant 4.9 ± 1.2 ng/μl, $p = 0.46$; plasma clusterin: younger onset 71.9 ± 11.1 ng/μl; tremor-dominant 72.0 ± 8.8 ng/μl; non-tremor dominant 74.5 ± 11.8 ng/μl; $p = 0.77$). Neither CSF nor plasma clusterin levels correlated with MMSE values (CSF clusterin in controls: $r = 0.15$, $p = 0.29$ and in PD: $r = -0.20$, $p = 0.15$; plasma clusterin in controls: $r = -0.06$, $p = 0.70$ and in PD: $r = -0.05$, $p = 0.72$).

In controls but not in PD patients, CSF clusterin levels positively correlated with plasma clusterin levels (controls: $r = 0.39$, $p = 0.005$;

**Table 1**
Demographics and CSF values.

| | Controls | Parkinson's disease | *p*-value |
|---|---|---|---|
| Number of cases | 50 | 52 | n.a. |
| Sex (M/F) | 16/34 | 32/20 | 0.003 |
| Age (years) | 63 ± 7 | 63 ± 10 | 0.75 |
| Disease duration (years) | n.a. | 4; 1–22 | n.a. |
| Hoehn and Yahr stage (number per stage 1/1.5/2/2.5/3/4/5) | n.a. | 3/4/23/16/6/0/0 | n.a. |
| UPDRS-III | n.a. | 22 ± 8 | n.a. |
| Clinical phenotype (younger onset/tremor-dominant/non-tremor dominant) | | 20/7/25 | n.a. |
| MMSE | 29; 25–30 | 29; 23–30 | 0.02 |
| CSF red blood cell count per μl | 1.5; 0–490 | 1; 0–499 | 0.25 |
| CSF clusterin (ng/μl) | 4.6 ± 1.1 | 4.9 ± 1.39 | 0.19 |
| Plasma clusterin (ng/μl) | 76.1 ± 12.6 | 73.2 ± 11.1 | 0.71 |
| CSF Aβ42 (ng/l) | 989 ± 214 | 926 ± 202 | 0.15 |
| CSF total tau (ng/l) | 218 ± 72 | 209 ± 87 | 0.35 |
| CSF phospho-tau (ng/l) | 41 ± 13 | 41 ± 16 | 0.86 |

Data are expressed as either mean ± SD or median and range unless specified otherwise. UPDRS-III = motor section of the Unified Parkinson's Disease Rating Scale.

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