

High Molecular Weight isoforms of DPP4 with distinct patterns in the serum of patients with Alzheimer's and Parkinson's Disease

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High-MW, native and enzymatically active forms of DPP4 in AD and PD patients compared to healthy individuals

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Covalently-linked DPP4 complexes with extremely high MW (148-190 kDa and above 245 kDa) in AD patients' serum.

Background: Dipeptidyl peptidase 4 (DPP4) is a multifunctional serine protease that cleaves N-terminal dipeptides adjacent to proline or alanine residues, thereby regulating the activity of multiple bioactive peptides, including glucagon-like peptide-1 (GLP-1), stromal cell-derived factor-1 (SDF-1), PACAP, brain natriuretic peptide (BNP), eotaxin, and substance P. Increasing evidence implicates DPP4 in neurobiological processes and neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). DPP4 has been shown to cleave amyloid- β ($A\beta$) peptides, generating N-terminally truncated forms ($A\beta_{3-x}$), which can subsequently undergo cyclization to form pyroglutamate-modified $A\beta$ (pE3- $A\beta$), a highly aggregation-prone and neurotoxic species. Additionally, through modulation of the GLP-1 axis, DPP4 regulates incretin signaling, with downstream effects on insulin signaling, neuroinflammation, and neuronal survival pathways that may contribute to neurodegenerative processes. Furthermore, DPP4 activity may influence synaptic function and neuroinflammation, partly through modulation of chemokine signaling and immune cell migration within the central nervous system. This study aimed to evaluate serum DPP4 isoform profiles in patients with AD and PD compared to healthy controls.

Methods: Serum samples were subjected to ion-exchange chromatography (DEAE) and size-exclusion chromatography (Sephacryl S-300) for isoform separation. Fractions were tested for DPP4 activity and were further characterized using SDS-PAGE and Western blot analysis. Using anti-DPP4 antibody, to assess molecular weight distribution.

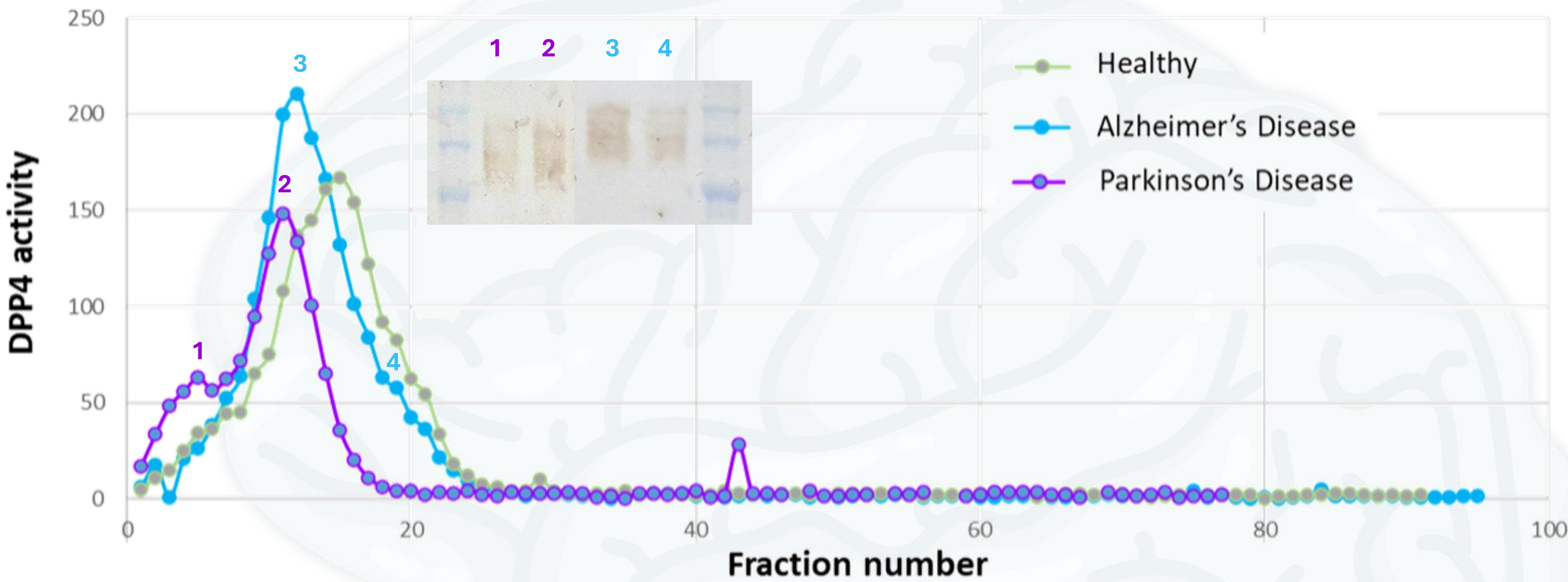


Figure 1. Size-exclusion chromatography (Sephacryl S-300) profile of DPP4-isoforms in the serum of Alzheimer's and Parkinson's Disease patients compared to healthy individuals. SDS-PAGE – Western blot of specific fractions.

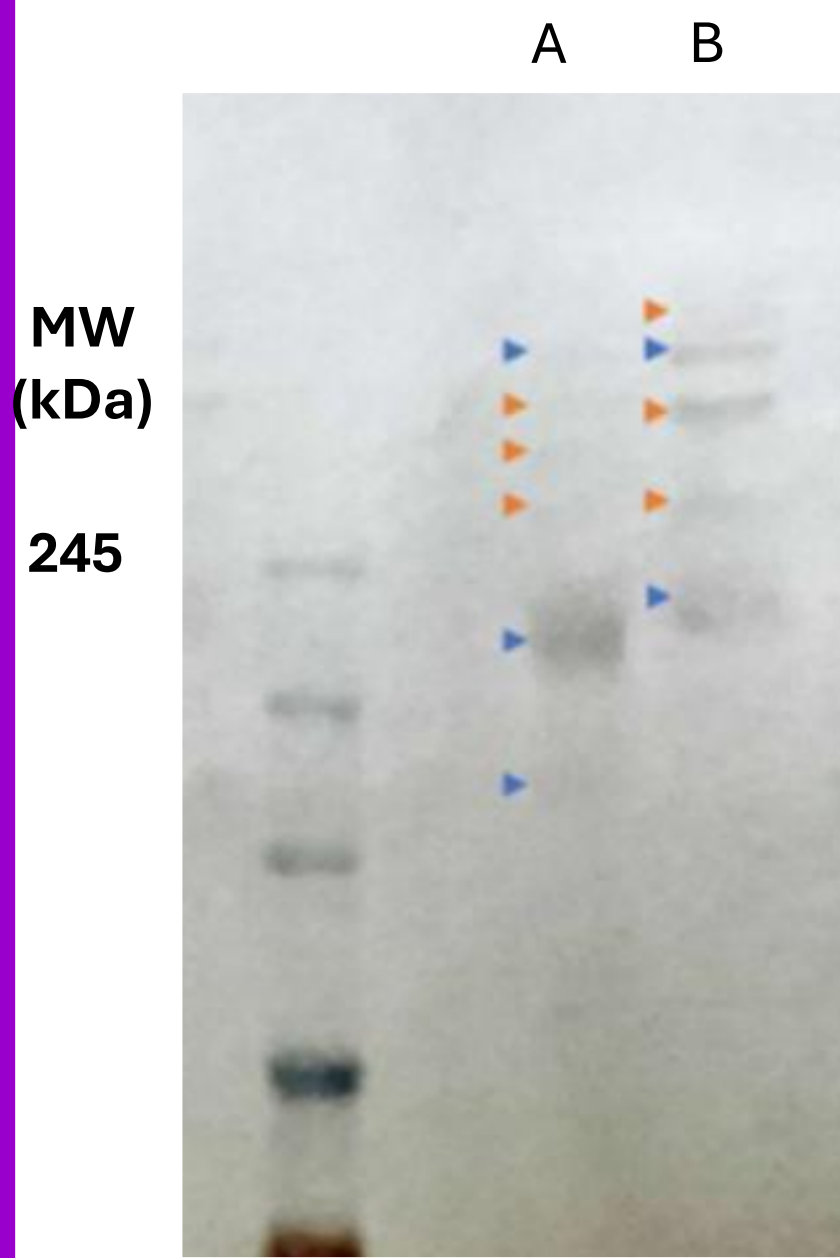


Fig.2. SDS-PAGE – Western blot of ion exchange chromatography fractions of AD patients' serum.

Results:

Size-exclusion chromatography revealed marked differences in molecular weight profiles in both AD and PD groups, with the presence of higher molecular weight, native, active isoforms in AD and PD patients compared to healthy individuals and distinct profile between the two diseases (Figure 1).

A higher MW group of forms with molecular mass varying between 148-190 kDa predominated in the higher activity S-300 fraction of AD compared to 117-165 kDa in the higher activity fraction of PD, as shown by SDS PAGE – Western blot analysis (Figure 1).

Apparently, in both patients, DPP4 isoforms are present as complexes involving covalent and non-covalent bonds with higher MW covalent moieties present in AD.

SDS-resistant (covalently linked) DPP4 complexes with extremely high MW (above 245 kDa) were also observed in DPP4 isoforms from AD patients' serum, separated by DEAE chromatography (Figure 2).

AD samples did not show significant shifts in the isoelectric point of DPP4 isoforms, while, in PD patients, slightly lower isoelectric points were observed compared to healthy controls, suggesting subtle charge alterations.

Conclusions:

In conclusion, both AD and PD patients displayed distinct serum DPP4 isoform patterns, characterized by high molecular mass forms.

These findings likely reflect, disease-associated post-translational modifications or protein complex formation and support a potential role for DPP4 as a biomarker and therapeutic target in neurodegenerative disorders.

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