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Cross-disease LC–MS/MS plasma proteomics identifies reproducible shared and disease-enriched biomarker signatures in neurodegenerative disorders

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Abstract

Neurodegenerative diseases (NDDs) exhibit considerable molecular heterogeneity, making it difficult to pinpoint robust, disease-specific biomarkers. Although proteomic studies have deepened our understanding of individual disorders, systematic cross-disease comparisons with cross-platform validation remain scarce,

especially for rare conditions like spinal and bulbar muscular atrophy (SBMA). To address this gap, we conducted a comparative plasma proteomic analysis using liquid chromatography–tandem mass spectrometry (LC–MS/MS) in 264 participants across major neurodegenerative and related diagnostic groups, including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), SBMA, and cognitively healthy controls. This unified framework allowed us to capture both disease-specific and shared protein signatures across neurodegenerative conditions. Candidate proteins were then validated in the UK Biobank (Olink Explore) and the Global Neurodegeneration Proteomics Consortium (SomaScan). Of 23 proteins assessed in the UK Biobank, four unique proteins (yielding six disease–protein associations) showed nominally significant and directionally concordant changes; of 20 proteins represented by 27 probes tested in the Global Neurodegeneration Proteomics Consortium, seven proteins reached nominal significance, all with full directional concordance across both cohorts. Notably, IGFBP2 was consistently elevated in AD and PD across independent datasets, pointing to shared metabolic dysregulation, while ADIPOQ showed parallel increases in the same conditions, reinforcing convergent shifts in energy metabolism. By contrast, CRTAC1 and COMP were selectively reduced in motor neuron diseases, suggesting disease-enriched alterations in extracellular matrix composition. Taken together, our findings provide a cross-disease, cross-platform framework for uncovering reproducible proteomic biomarkers and shed light on both overlapping and distinct molecular pathways in neurodegeneration.

Keywords:

Neurodegenerative diseases, Plasma proteomics, LC–MS/MS, Biomarkers, Cross-platform validation, UK Biobank, Global Neurodegeneration Proteomics Consortium

Introduction

Neurodegenerative diseases (NDDs) are a heterogeneous group of neurological disorders characterized by the progressive loss of neurons in the central nervous system (CNS) and peripheral nervous system (PNS), causing symptoms such as memory loss, neuropsychiatric manifestations, motor dysfunction, and profound disability [1]. Alzheimer's disease (AD) affects approximately 55 million people worldwide, Parkinson's disease (PD) affects 8.5 million, while motor neuron diseases including amyotrophic lateral sclerosis (ALS) and spinal and bulbar muscular atrophy (SBMA) are rarer but devastating conditions. The growing global prevalence of NDDs presents a significant socioeconomic burden [2].

The heterogeneous nature of neurodegenerative diseases, along with their complex pathophysiology, necessitates the development of sensitive and specific biomarkers for early detection, accurate disease staging, effective patient stratification, and monitoring of therapeutic responses. Conventional diagnostic techniques primarily rely on clinical symptoms and neuroimaging, which typically manifest after considerable neuronal loss has occurred [3]. These limitations underscore the need for molecular biomarkers that can detect presymptomatic pathology and guide therapeutic decision-making.

Recently, proteomics has emerged as a transformative approach for understanding NDDs, offering direct molecular profiling of proteins, their isoforms, and post-translational modifications in disease-relevant biological materials [4, 5]. Liquid chromatography-mass spectrometry (LC/MS) proteomics combines broad discovery capacity with quantitative precision of targeted quantitation. This integration allows for the characterization of thousands of proteins while retaining the sensitivity needed to detect post-translational modifications and low-abundance biomarkers [6]. Plasma proteomics is well suited for large-scale screening given its minimal invasiveness, though circulating protein levels may not fully capture CNS-specific changes. Cerebrospinal fluid offers a more proximal measure of brain pathology, but the invasiveness of lumbar puncture limits its broader application [7, 8].

Blood biomarkers show great potential for diagnosing NDDs, however standardized methods and validation in diverse populations are essential for achieving reproducible results in research and clinical settings [9-

11]. In addition, plasma proteomics offers several advantages, including minimal invasiveness, ease of collection, and the potential for repeated sampling to monitor disease progression and treatment response [12, 13].

Recent large-scale consortia have started to chart proteomic signatures across neurodegenerative diseases. For example, the Global Neurodegeneration Proteomics Consortium (GNPC) analyzed more than 35,000 biofluid samples from individuals with AD, PD, FTD, and ALS, uncovering distinct patterns of protein overlap—showing a strong PD–FTD association but very little overlap between AD and PD [14]. Similarly, the UK Biobank (UKB) plasma proteomics initiative has linked proteomic variation to health outcomes in more than 50,000 individuals [15]. Still, SBMA has been absent from all these large-scale investigations.

SBMA (Kennedy disease) is a rare X-linked motor neuron disorder arising from CAG repeat expansion in the androgen receptor gene, affecting roughly 1–2 per 100,000 males [16]. It overlaps clinically with ALS in presenting progressive weakness and muscle atrophy, yet differs in its slower progression and distinct pathogenesis driven by androgen receptor toxicity [17, 18]. No prior study has placed the SBMA plasma proteome alongside those of AD, PD, and ALS in a unified analytical framework, leaving this orphan disease largely absent from cross-disease proteomic comparisons [19, 20].

Although proteomic studies have significantly advanced our understanding of individual neurodegenerative diseases, a critical knowledge gap persists regarding the convergent and divergent molecular signatures across these conditions. Most proteomic studies have primarily focused on individual diseases, limiting the ability to systematically distinguish disease-specific from shared molecular signatures across neurodegenerative disorders. In addition, variability across analytical platforms may affect the reproducibility and generalizability of candidate biomarkers. Elucidating these shared and disease-specific protein alterations is paramount, holding profound implications for the development of differential biomarkers and novel therapeutic strategies. Specifically, unique protein signatures can inform the design of targeted therapies, whereas common molecular pathways may unveil broadly applicable therapeutic

targets. Furthermore, a deeper understanding of the molecular interplay between these disorders is essential for refining patient stratification and monitoring treatment outcomes.

In this study, we conducted a comprehensive, multi-disease comparative proteomic analysis spanning a broad spectrum of neurodegenerative disorders using standardized LC–MS/MS methodologies. Plasma proteins were systematically profiled from a diverse cohort comprising patients diagnosed with AD, PD, ALS, and SBMA, along with cognitively healthy controls (HCs). In the discovery phase, we performed label-free quantitative LC–MS/MS to identify both disease-specific and shared proteomic signatures, applying stringent statistical criteria to control for multiple comparisons. To evaluate whether these signatures generalize beyond our discovery cohort, we carried out independent validation in large-scale populations from the UKB and GNPC, each profiled on a different proteomic platform. This cross-platform validation approach was intended to prioritize biomarker candidates with consistent effect directions, delineate convergent pathological pathways, and identify clinically relevant biomarker candidates across the neurological disorders examined.

Materials and methods

Standard protocol approvals, registrations, and participants consent

All participants underwent clinical evaluation at the Neurodegenerative Disease Center of Kyungpook National University Chilgok Hospital (KNUCH) during the period from 2020 to 2023. Written informed consent was obtained from each participant and, where applicable, from their legally authorized caregivers prior to enrollment. Clinical diagnoses were established by neurologists at the Neurodegenerative Disease Center of Kyungpook National University Chilgok Hospital according to disease-specific diagnostic guidelines. Patients with Alzheimer's disease were selected on the basis of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable or possible AD [21], and patients with mild cognitive impairment were selected according to the Petersen criteria [22]. Additional evaluations, including neurological examination, laboratory blood tests, and CT or MRI, were performed to exclude other causes of dementia. Amyloid status was subsequently confirmed by florbetaben PET using the brain beta-amyloid plaque load (BAPL) scoring system, with amyloid-positive cases defined as BAPL 2 or 3 and amyloid-negative cases as BAPL 1 [23]; this allowed amyloid-positive MCI (ADMCI) and amyloid-negative MCI to be distinguished. Patients with Parkinson's disease met the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria [24]. These diagnoses were confirmed using [^{18}F]fluoro-L-DOPA (^{18}F -DOPA) positron emission tomography (PET), which demonstrated reduced dopaminergic uptake in the posterior putamen. Patients with spinal and bulbar muscular atrophy were genetically confirmed, carrying more than 38 CAG repeats in the first exon of the androgen receptor gene [25], and patients with amyotrophic lateral sclerosis met the probable or clinically definite category of the revised El Escorial criteria [26]. The study protocol was reviewed and approved by the Institutional Review Board of KNUCH (approval number: KNUMC-2023–01-036-001). In this dementia infrastructure project, a total of 270 patient samples were collected and analyzed using LC–MS/MS for proteomic profiling. Among the 264 successfully normalized samples, the cohort included cognitively HCs ($n = 58$), SBMA ($n = 42$), AD ($n = 34$), PD ($n = 30$), mild

cognitive impairment with amyloid PET positivity (ADMCI, $n = 20$), amyloid-negative mild cognitive impairment (MCI, $n = 20$), and ALS ($n = 20$). Cognitive and functional status was characterized using a multidimensional battery rather than a single global measure. Cognitive screening and staging relied on the Korean Mini-Mental State Examination (K-MMSE), the Global Deterioration Scale (GDS), and the Clinical Dementia Rating (CDR), and the CDR Sum of Boxes (CDR-SB) was used to capture the more graded changes prominent in mild cognitive impairment and early-stage disease. Functional independence was assessed with the Bayer Activities of Daily Living (B-ADL) and the Korean Instrumental Activities of Daily Living (K-IADL) scales, which together verify whether cognitive decline interfered with daily functioning, a criterion used to separate mild cognitive impairment from dementia. For patients with Parkinson's disease, motor severity was additionally rated using the Unified Parkinson's Disease Rating Scale (UPDRS), the Hoehn and Yahr scale (H&Y), and Schwab and England Activities of Daily Living (SEADL). For patients with SBMA and ALS, motor function was assessed using the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) and the 6-Minute Walk Test (6MWT). Demographic and clinical characteristics of participants are shown in Table 1 and Supplementary Table 1.

Less frequent diagnostic categories—representing samples that were reclassified after the initial blood collection based on supportive neuroimaging findings (such as routine structural brain MRI or clinical PET scans) and longitudinal follow-up criteria—included dementia with early AD pathology (BAPL2, $n = 8$), motor neuron disease (MND, $n = 6$), vascular dementia (VaD, $n = 4$), scans without evidence of dopaminergic deficit (SWEDD, $n = 3$), normal pressure hydrocephalus (NPH, $n = 3$), AD with comorbid NPH (AD/cNPH, $n = 5$), atypical parkinsonism with comorbid NPH (APD/cNPH, $n = 2$), hereditary spastic paraplegia (HSP, $n = 2$), sporadic adult-onset ataxia (SAOA, $n = 2$), dementia with Lewy bodies (DLB, $n = 1$), frontotemporal lobar degeneration (FTLD, $n = 1$), vascular parkinsonism (VaP, $n = 1$), and unknown diagnosis ($n = 2$).

The sample composition underscores the heterogeneity of neurodegenerative diseases and provides a comprehensive basis for comparative proteomic analyses. The inclusion of both common and rare dementia

subtypes strengthens the potential of this dataset to contribute to the understanding of disease-specific biomarkers and pathophysiological mechanisms.

Mass Spectrometry Analysis for Proteomics

Sample Preparation

Albumin and IgG depletion from human plasma samples was performed using High Select™ Depletion Spin Columns (Thermo Fisher Scientific, A36366). The depletion spin columns were equilibrated to room temperature for 30 min, and up to 10 μ L of sample was directly added to the resin slurry within the column. The resin was fully homogenized and incubated with gentle end-over-end mixing for 10 min at room temperature. After incubation, the mini column was placed into a 2 mL collection tube and centrifuged at $1000 \times g$ for 2 min. The resin-containing column was removed and discarded. The flow-through, containing the sample with albumin and IgG removed, was buffer-exchanged using Vivaspin® 500 centrifugal concentrators with a 3 kDa molecular weight cut-off (MWCO) (Sartorius, VS0192). The concentrator was first washed with 450 μ L of 40 mM ammonium bicarbonate (ABC) buffer at $15,000 \times g$ for 30 min at 20°C. The sample was then loaded into the concentrator and centrifuged at $15,000 \times g$ for 30 min at 20°C. Buffer exchange was performed by adding 450 μ L of 40 mM ABC buffer to the concentrated sample and centrifuging again under the same conditions. A final back-spin was carried out at $3,000 \times g$ for 2 min at 20°C. Protein concentration was determined using the bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, 23225), and 40 μ g of protein was prepared for downstream analysis.

In-Solution Digestion

In-solution digestion was performed using Trypsin/Lys-C Mix (Promega, V5073). To reduce disulfide bonds, 10 mM dithiothreitol (DTT) was added to the 40 μ g protein sample and incubated at 56°C for 20 min. Subsequently, 20 mM iodoacetamide (IAA) was added and the mixture was incubated in the dark at room temperature for 20 min to allow alkylation. Then, 2 μ g of Trypsin/Lys-C Mix (enzyme-to-protein ratio of 1:50, w/w) was added. The sample was digested overnight (~18 h) at 37°C, followed by

centrifugation at $16,000 \times g$ for 10 s at 4°C to collect the supernatant.

Desalting and preparation for LC–MS/MS

Desalting was performed using Pierce™ C18 spin columns. The C18 resin was first activated by washing with 50% methanol and then equilibrated with the equilibration buffer. The peptide sample was loaded onto the column, and unbound salts and contaminants were removed with a wash buffer. Desalted peptides were eluted with elution buffer, collected, and vacuum-dried at 35°C and 10 mbar for 4 h. For mass spectrometry analysis, the dried eluate was reconstituted in 20 µL of 0.1% formic acid in HPLC-grade water, vortexed at 25°C, and a 10 µL aliquot was injected into the LC–MS/MS system.

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis

Peptide analysis was performed using a Q-Exactive™ Plus Hybrid Quadrupole–Orbitrap mass spectrometer coupled with an EASY-Spray™ ion source (Thermo Fisher Scientific). Chromatographic separation was carried out on an UltiMate™ 3000 RSLC nano system (Thermo Fisher Scientific) equipped with an Acclaim™ PepMap™ 100 C18 trap column (75 µm × 2 cm, 3 µm, nanoViper; Thermo Fisher Scientific) and an EASY-Spray™ PepMap™ RSLC C18 analytical column (75 µm × 50 cm, 2 µm; Thermo Fisher Scientific). Peptides were injected via an RS autosampler and separated using a linear gradient of acetonitrile in water (both containing 0.1% formic acid) at a flow rate of 0.3 µL/min. The eluate was electrosprayed from the analytical column under a 2.0 kV potential applied through the nanospray source. Peptide separation was performed with a 10–50% acetonitrile gradient over 80 min. Data acquisition included a full MS scan over the range of m/z 350–2000, followed by data-dependent MS/MS analysis of the 10 most intense precursor ions from the MS1 scan. The instrument was operated in data-dependent acquisition (DDA) mode. Mass spectrometer calibration was conducted using the recommended calibration solution according to the manufacturer's instructions.

Protein quantification using MaxQuant

All mass spectrometry raw files were processed using MaxQuant software (version 2.4.10.0) [27, 28]. The

MS/MS spectra were searched against the reviewed human proteome FASTA file from the UniProtKB database containing 20,601 non-redundant sequences, supplemented with the MaxQuant contaminant database to account for common laboratory contaminants.

Protein digestion was performed in silico using Trypsin, allowing a maximum of two missed cleavages. Label-free quantification (LFQ) was carried out using the MaxLFQ algorithm [29], with the "match between runs" (MBR) option enabled, allowing quantification of MS1 features that were not identified in every single run [28].

The false discovery rate (FDR) was set at 1% (0.01) at both the peptide and protein levels. Peptides with a minimum length of seven amino acids were required for identification, and FDR was determined by searching against a reversed decoy database. The first search mass tolerance and main search mass tolerance were set at 20 ppm and 4.5 ppm, respectively.

Fixed modifications included cysteine carbamidomethylation (+57.0215 Da), while variable modifications were set as protein N-terminal acetylation (+42.0106 Da), asparagine and glutamine deamidation (+0.9840 Da), and methionine oxidation (+15.9949 Da). Enzyme specificity was defined as cleavage C-terminal to arginine and lysine residues, consistent with trypsin digestion.

After log₂ transformation of LFQ intensity values, missing values were imputed using a left-censored normal distribution (width = 0.3, downshift = 1.8 standard deviations), a widely adopted approach in label-free proteomics to approximate low-abundance signals below the detection limit [30, 31]. Although sensitivity analyses with alternative imputation strategies were not performed, the external replication of key findings in UKB and GNPC provides indirect support for the robustness of these results. After removing known contaminants, the protein intensity profiles from all diagnostic groups—including HC, AD, ADMCI, PD, SBMA, ALS, MCI and others—were merged into a single dataset. Median intensity normalization was applied across all samples to correct for global intensity differences and enable fair comparisons between disease and control groups.

Differential Expression Analysis

To identify differentially expressed proteins (DEPs) between HCs and the distinct disease groups, we performed statistical comparisons using the Mann-Whitney U test (Figure 1). This non-parametric method was selected to accommodate the small sample sizes ($n < 30$) in the AD, ADMCI, and ALS groups.

Following the initial identification of DEPs, a subsequent analysis involving pairwise group comparison using Mann-Whitney U tests was conducted across multiple combinations of diagnostic groups (Figure 2, 3). This approach aimed to distinguish proteins that were: predominantly associated with a single disease (i.e., AD-predominant, PD-predominant, ALS-predominant, or SBMA-predominant), or commonly altered between related conditions (i.e., common to both AD and PD, or to both ALS and SBMA). Statistical significance for all tests was defined as a p -value less than 0.05. All data analysis and graphical representation were performed in the R software environment (version 4.5.0). Given the exploratory nature of our discovery analysis with modest sample sizes, we applied a nominal significance threshold of $p < 0.05$ without multiple testing correction, recognizing that this approach may inflate the false positive rate. External validation in UKB and GNPC was therefore used as a confirmation step to mitigate this limitation. In the discovery cohort, we did not adjust for age and sex due to the limited sample size, which would have reduced statistical power. However, covariate-adjusted analyses were performed in the larger validation cohorts to assess whether the observed associations were independent of demographic factors.

External Validation Cohorts and Statistical Analysis

To determine whether the protein signatures identified in our LC-MS/MS analysis extend to broader populations, we leveraged two independent external datasets: the UKB and the GNPC. The UKB dataset comprises plasma proteomic profiles from approximately 53,000 participants, measured on the Olink Explore 3072 platform [15], while the GNPC dataset includes plasma proteomic data from multiple neurodegenerative disease cohorts profiled on the SomaScan 7K platform [14]. Sample sizes for each diagnostic group are summarized in Supplementary Table 2. Given that the datasets were generated on

different analytical platforms, protein identifiers were harmonized by mapping Olink UniProt IDs and SomaLogic sequence IDs to standardized gene symbols, enabling direct cross-platform comparison.

For the GNPC cohort, we applied stricter exclusion criteria than those used in prior GNPC publications, where various inflammatory conditions had been permitted in control groups. To enhance phenotypic specificity and minimize the influence of non-neurodegenerative inflammatory signals, individuals with a documented history of other neurodegenerative diseases, stroke, TIA, TBI, or cancer were excluded from all disease groups. Only the most recent visit per participant was retained to reduce the impact of diagnostic changes over time. HCs were further restricted to individuals free of metabolic or psychiatric disorders, as determined by demographic records and ICD-10 codes, and were required to have a CDR score of 0 alongside cognitively normal MMSE or MoCA performance. Group-level differences in protein abundance were tested using ANCOVA, adjusting for age, sex, and cohort, in line with established GNPC analytical frameworks [32, 33].

For the UKB cohort, analyses were restricted to participants aged 60 years or older, given that the UKB population skews younger than typical neurodegenerative disease cohorts. Disease cases were defined using broadened diagnostic criteria that included diagnoses made after the initial blood draw, thereby capturing both clinical and prodromal stages. HCs were strictly defined by excluding individuals with any neurological, metabolic, inflammatory, or psychiatric condition based on ICD-10 codes and available clinical metadata. Protein-level differences between groups were assessed using ANCOVA adjusted for age and sex [15, 34].

Following the study by Imam et al. [14], we adopted standardized coefficients to measure the magnitude of effects and the direction of protein expression alterations (i.e., up- or down-regulation). This approach was particularly motivated by the multi-cohort structure of the GNPC dataset, where scaling and normalization differences across cohorts can confound direct comparisons based on raw fold changes. For methodological consistency, the same approach was also applied to the UKB dataset.

By examining these independent cohorts across distinct proteomic platforms, we aimed to evaluate the reproducibility and generalizability of the candidate biomarkers identified in our discovery analysis.

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Results

Identification of Differentially Expressed Proteins Across Neurodegenerative Diseases

To quantify and compare plasma protein levels across all samples, raw data were first processed using MaxQuant software with the MBR algorithm, rather than relying on instrument-provided quantification as described in Methods. Median intensity normalization was then applied to generate a final expression matrix suitable for cross-disease comparisons.

To provide an overview of proteomic alterations across diagnostic groups, we performed pairwise comparisons between each neurodegenerative disease cohort and cognitively normal controls, and visualized the results as volcano plots (Figure 1A–F). Each plot displays $\log_2$ fold changes on the x-axis against statistical significance on the y-axis, highlighting proteins significantly upregulated (red) or downregulated (blue) at $p < 0.05$. This approach allowed us to capture both the breadth and direction of protein alterations in each condition.

Overall, the extent of proteomic dysregulation varied substantially across diseases. SBMA (Figure 1F) showed the most pronounced differences, with 39 differentially expressed proteins (11 upregulated and 28 downregulated), whereas amyloid-negative MCI (Figure 1C) displayed the fewest alterations. AD, PD, and ALS demonstrated intermediate numbers of significant proteins, each with distinct directional biases. These differences should be interpreted with caution, as the number of DEPs identified in each panel of Figure 1 depends strongly on group size and the associated statistical power, rather than on the underlying biology alone. Smaller cohorts such as ALS ($n = 20$) and AD ($n = 34$) offer limited power to reach significance, whereas larger cohorts such as SBMA ($n = 42$) yield more DEPs at a comparable effect size. The 39 DEPs observed in SBMA therefore likely reflect this power advantage in part, rather than a uniquely greater degree of proteomic dysregulation. Across all groups, the DEP counts in Figure 1 should be regarded as power-dependent, and studies with balanced sample sizes will be required to compare the true extent of dysregulation across diseases.

Figure 1 highlights both the heterogeneity and the presence of common protein alterations across neurodegenerative disorders. Some proteins exhibited the most pronounced alterations in specific disease groups compared with HC, such as LPA in AD, CFHR1 in PD, F13B in ALS, and CRTAC1 in SBMA. Other proteins, including ADIPOQ (elevated in AD, ADMCI, and PD) and APOC2 (reduced in both AD and PD), were altered across multiple groups, suggesting shared biological mechanisms. Therefore, it is important to distinguish DEPs that are disease-specific from those that are shared, by systematically comparing each group with HC and across disease groups.

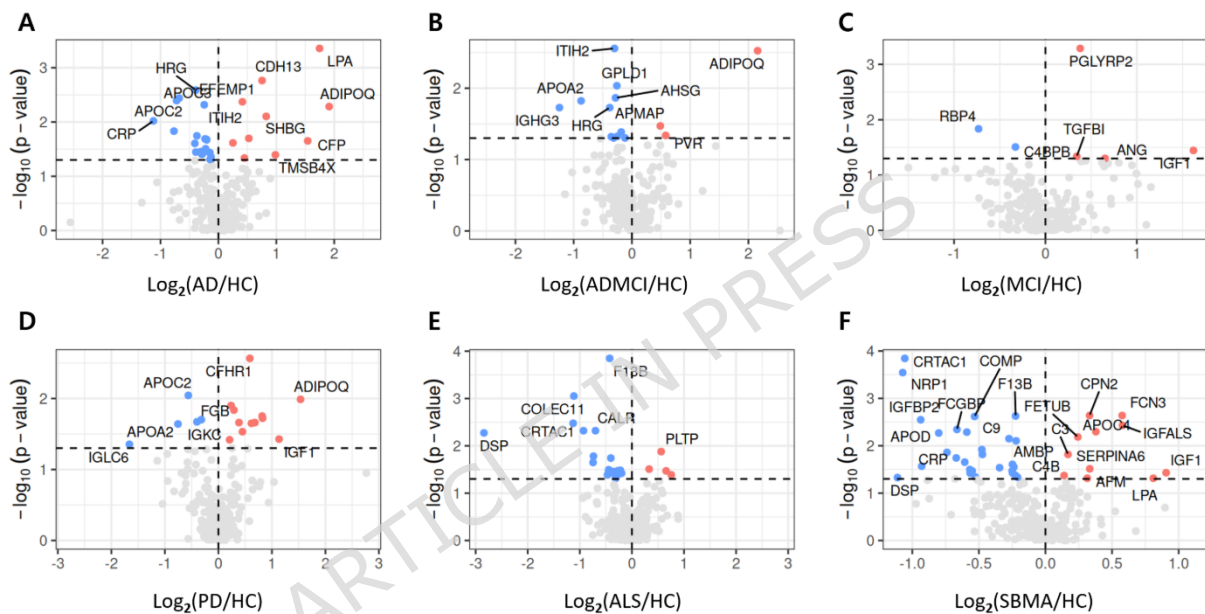


Figure 1. Volcano plots of differentially expressed plasma proteins across neurodegenerative disease groups. Each plot (A–F) shows $\log_2$ fold change versus $-\log_{10}(p)$, with proteins significantly upregulated ($p < 0.05$) in red and downregulated in blue (Mann-Whitney U test). Labeled proteins represent those with the largest and most significant changes. Conditions: (A) Alzheimer’s disease (AD, $n = 34$ vs HC, $n = 58$), (B) amyloid-positive MCI (ADMCI, $n = 20$), (C) amyloid-negative MCI (MCI, $n = 20$), (D) Parkinson’s disease (PD, $n = 30$), (E) amyotrophic lateral sclerosis (ALS, $n = 20$), and (F) spinal and bulbar muscular atrophy (SBMA, $n = 42$). This comparative analysis highlights both disease-specific and shared proteomic alterations that may reflect underlying pathophysiological processes.

Characterization of AD- and PD-Specific Protein Signatures

The heatmaps in Figure 2 summarize cross-disease comparisons of DEPs derived from AD vs HC (Figure 2A) and PD vs HC (Figure 2B). In these analyses, proteins identified as DEPs in the initial AD vs HC or PD vs HC comparisons ($p < 0.05$) were systematically tested against additional disease groups, including ADMCI, MCI, ALS, SBMA, and several combined cohorts (AD+ADMCI, AD+PD, AD+ADMCI+PD, ALS+SBMA). This approach allowed us to evaluate whether the expression differences observed in AD and PD were preserved when compared to other conditions, thereby clarifying their disease specificity.

Proteins predominantly associated with AD (red labels) were defined as those significant in AD vs HC and in at least two of three key comparisons (AD vs PD, AD vs ALS, AD vs SBMA). Likewise, PD-predominant proteins (blue labels) were defined as those significant in PD vs HC and in at least two of three corresponding comparisons (PD vs AD, PD vs ALS, PD vs SBMA). Proteins classified as AD & PD common (purple labels) were required to be DEPs in both AD vs HC and PD vs HC and to show differences in at least one of the two additional comparisons against ALS or SBMA.

The heatmaps highlight several examples of disease-predominant alterations. In AD, CDH13 showed consistent differences compared with MCI, PD, and SBMA, but not ALS (Figure 2A). EFEMP1, SHBG, ITIH1, ITIH2, and KLKB1 also exhibited significant changes in AD relative to ALS and SBMA, although these were not uniformly observed in comparisons with PD. In PD, IGF2 was robustly upregulated compared with AD, ALS, and SBMA (Figure 2B), while CFHR1 and LYZ were significantly increased in PD compared with AD and SBMA, but showed no consistent differences relative to ALS.

In addition to these disease-specific proteins, several DEPs displayed shared patterns across AD and PD. ADIPOQ and IGFBP2 were consistently upregulated in both diseases relative to HC and SBMA, whereas APOC2 was uniformly downregulated in both AD and PD compared with HC. These common alterations suggest overlapping biological mechanisms, particularly in metabolic and hormonal regulation. By contrast, disease-predominant proteins may reflect distinct processes, such as lipid metabolism and extracellular matrix remodeling in AD, or innate immune activation and growth factor signaling in PD. Together, these results illustrate how cross-disease comparisons can disentangle proteins with strong disease specificity.

from those reflecting broader, shared mechanisms of neurodegeneration. These AD- and PD-related protein signatures are summarized in Table 2.

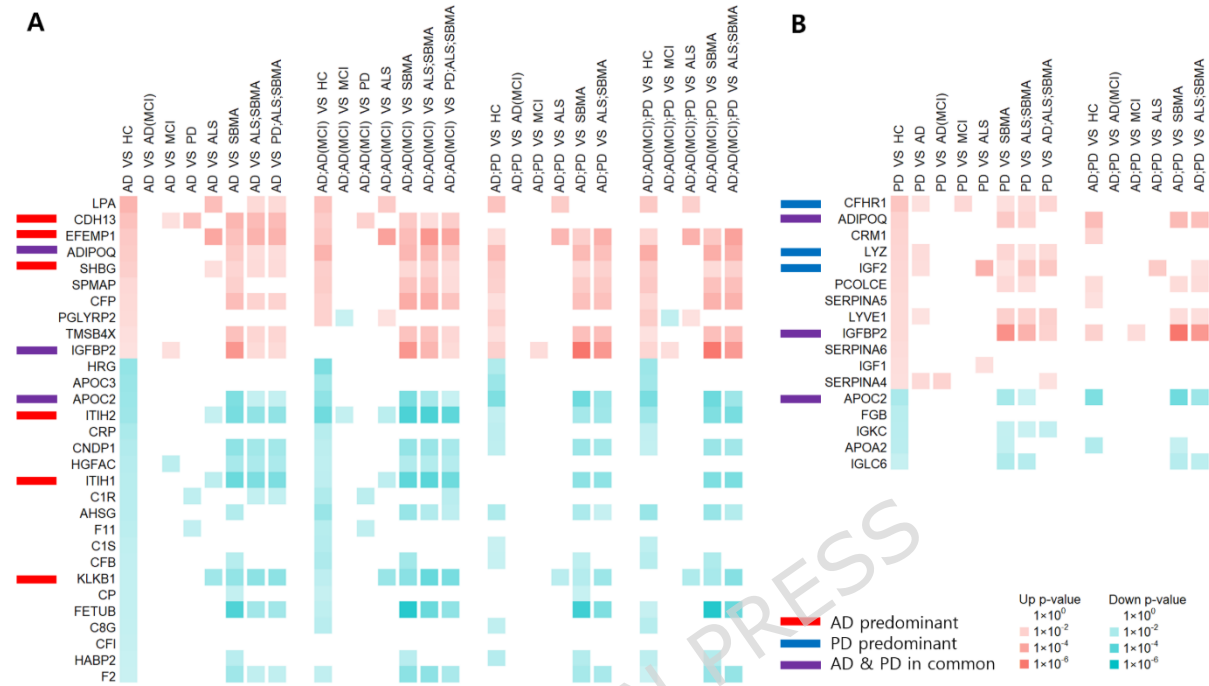


Figure 2. Disease specificity of DEPs in AD and PD. (A) DEPs derived from AD vs HC and tested against additional disease groups (ADMCI, MCI, ALS, SBMA) and combined cohorts (AD+ADMCI, AD+PD, AD+ADMCI+PD, ALS+SBMA). (B) DEPs derived from PD vs HC tested in the same manner. Proteins on the y-axis are ordered by direction of alteration (upregulated at the top, downregulated at the bottom) and sorted by initial p-values. Each tile represents the p-value from a Mann-Whitney U test, with red indicating upregulation and blue indicating downregulation; darker colors denote stronger significance. Labels on the y-axis denote AD-predominant (red), PD-predominant (blue), or AD & PD common (purple) proteins, as defined in the text.

Distinct and Shared Proteomic Signatures in Motor Neuron Diseases

To further investigate the molecular signatures of motor neuron diseases, we extended our cross-disease analysis to proteins associated with ALS and SBMA. DEPs initially identified from ALS vs HC and SBMA vs HC comparisons ($p < 0.05$) were systematically tested against other neurodegenerative conditions, including AD, ADMCI, MCI, and PD, as well as combined cohorts, to evaluate whether their differential expression patterns were preserved (Figure 3).

Proteins predominantly associated with ALS (red labels) were defined as those significant in ALS vs HC and in at least two of three key comparisons (ALS vs AD, ALS vs PD, ALS vs SBMA). Likewise, SBMA-predominant proteins (blue labels) were defined as those significant in SBMA vs HC and in at least two of three corresponding comparisons (SBMA vs AD, SBMA vs PD, SBMA vs ALS). Proteins classified as ALS & SBMA common (purple labels) were required to be DEPs in both ALS vs HC and SBMA vs HC and to show significant differences in at least one of the additional comparisons against AD or PD.

The heatmaps highlight distinct patterns of protein dysregulation for each motor neuron disease. In ALS, CDH13 was upregulated compared with SBMA and PD but not AD (Figure 3A), while C4BPB was significantly increased in ALS compared with both AD and PD. Conversely, CALR, CPB2, EFEMP1, and CSF1R were consistently downregulated in ALS. Notably, EFEMP1 displayed opposite regulation in ALS (downregulated) versus AD (upregulated), suggesting divergent pathological mechanisms between the two diseases.

Among the four disease groups, SBMA exhibited the most distinct proteomic profile. A defined set of disease-predominant proteins—FETUB, AFM, IGFBP2, CFHR5, and FBLN1—showed significant alterations in SBMA relative to AD, PD, and ALS (Figure 3B). Of note, FETUB is a liver-derived plasma glycoprotein with established roles in insulin resistance and metabolic homeostasis. Its selective dysregulation in SBMA is of particular interest given that metabolic dysfunction, including impaired glucose tolerance and altered lipid metabolism, is a recognized clinical feature of the disease. Similarly, AFM, a vitamin E-binding glycoprotein involved in antioxidant transport, was selectively reduced in SBMA, possibly reflecting oxidative stress-related mechanisms that are more prominent in SBMA pathogenesis than in the other neurodegenerative conditions examined here.

Beyond these SBMA-specific changes, a shared proteomic signature was observed across the two motor neuron diseases. F13B, CRTAC1, and COMP were consistently decreased in both ALS and SBMA when compared with AD and PD. This convergent pattern of downregulation suggests the existence of common pathological mechanisms—potentially related to extracellular matrix remodeling or coagulation

pathways—that differentiate motor neuron diseases from other forms of neurodegeneration. These ALS- and SBMA-related protein signatures are also collectively summarized in Table 2.

Two features of these motor neuron diseases differ in a way that matters for interpretation. In ALS, muscle involvement largely follows lower motor neuron loss. In SBMA the situation is reversed, and skeletal muscle is increasingly viewed as a primary, early site of disease [35, 36]. Animal work makes the same point. Muscle pathology appears before spinal cord changes [37], confining mutant androgen receptor expression to muscle is enough to reproduce the phenotype [38], and silencing the receptor peripherally rescues it [39]. COMP and CRTAC1 are extracellular-matrix glycoproteins, and their plasma levels move with connective-tissue and cartilage turnover [40]. Their decline in ALS and SBMA may then come partly from muscle and connective tissue rather than from a shared neuronal process, which is especially worth keeping in mind for SBMA.

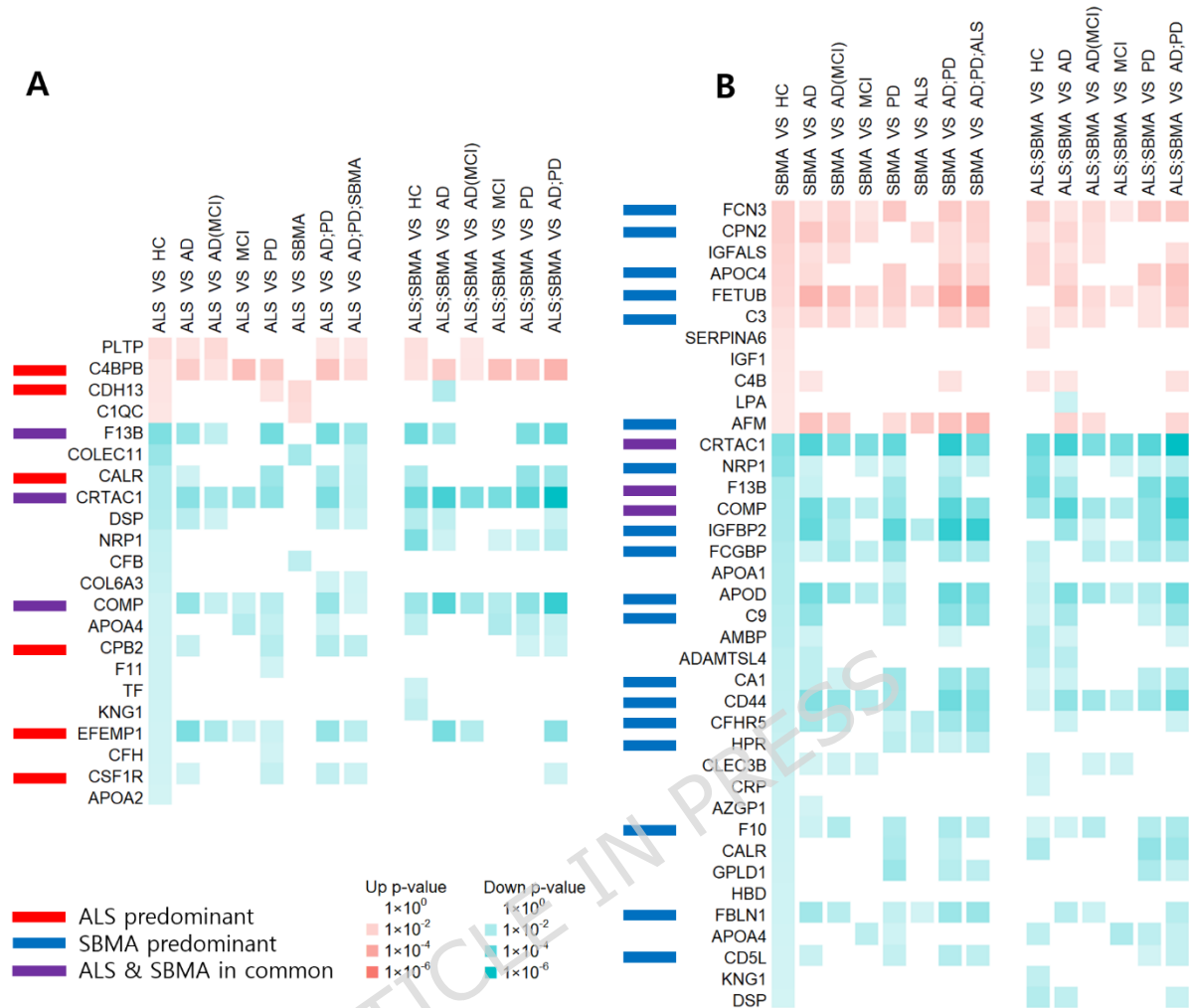


Figure 3. Disease specificity of DEPs associated with ALS and SBMA. Heatmaps showing cross-disease comparison of DEPs derived from ALS vs HC. (A) DEPs derived from ALS vs HC tested against additional disease groups (AD, ADMCI, MCI, PD) and combined cohorts. (B) DEPs derived from SBMA vs HC tested in the same manner. Proteins on the y-axis are ordered by direction of alteration (upregulated at the top, downregulated at the bottom) and sorted by initial p-values. Each tile represents the p-value from a Mann-Whitney U test, with red indicating upregulation and blue indicating downregulation; darker colors denote stronger significance. Labels on the y-axis denote ALS-predominant (red), SBMA-predominant (blue), or ALS & SBMA common (purple) proteins, as defined in the text.

Validation using Olink-based UK Biobank Data

To explore the broader applicability of our LC–MS findings, we examined the UKB dataset generated using the Olink Explore 3072 platform. Among the proteins identified in our LC–MS discovery analysis, 23

proteins were measurable in the UKB Olink dataset. As these candidates were identified as disease-specific biomarkers in the LC–MS analysis, each protein was tested within its corresponding disease group by comparing patients with HCs. Statistical differences were evaluated using ANCOVA adjusted for age and sex (Table 3; Supplementary Table 3).

Of these 23 proteins, four showed statistically significant differences between disease groups and HCs based on nominal p-values. Importantly, all four proteins exhibited directional changes consistent with those observed in the LC–MS dataset, supporting the robustness of these candidates across independent cohorts and analytical platforms.

We first evaluated the shared protein signatures identified for AD and PD. In the UKB cohort, ADIPOQ and IGFBP2 were significantly elevated in both AD and PD compared with HCs, mirroring the upregulation observed in our LC–MS analysis. The consistent increase of these proteins across platforms suggests that they may reflect shared biological mechanisms underlying neurodegenerative processes.

We next examined disease-specific candidates. For AD, KLKB1 showed a significant decrease in the UKB dataset, reproducing the downregulation observed in the LC–MS discovery cohort. This cross-platform replication supports the potential of KLKB1 as an AD-associated biomarker.

In the UKB cohort, MND cases—defined as per ICD-10 codes encompassing ALS and related conditions—were analyzed as a single group due to limited diagnostic granularity. CRTAC1 demonstrated a significant reduction in patients, consistent with the direction observed in our discovery phase results. The replication of this decrease in a large population-based cohort further suggests that CRTAC1 may reflect molecular changes associated with motor neuron degeneration.

Although only a subset of candidate proteins reached nominal statistical significance in the UKB analysis, the consistent direction of change observed for these proteins across independent cohorts and distinct proteomic platforms provides supportive evidence for the robustness and cross-platform reproducibility of these biomarker candidates.

Validation using SomaLogic-based GNPC Data

To further assess the consistency of our findings, we utilized the GNPC database, which is based on the SomaScan 5K/7K platform. For this analysis, we adjusted for age, sex, and cohort using ANCOVA to account for potential variations across different sample groups (Table 4; Supplementary Table 4). Among the candidate proteins identified in our LC–MS discovery analysis, a total of 20 proteins (represented by 27 SomaScan probes) were evaluated in the GNPC dataset. Of these, seven proteins reached nominal significance in the GNPC, and each moved in the same direction as in our LC–MS data. We keep two ideas apart here. A protein passed validation only when it was significant with a matching direction; when the direction matched but significance was not reached, we call it directionally concordant and nothing more. CRTAC1, KLKB1, ADIPOQ, and IGFBP2 cleared the higher bar in both cohorts. SHBG, IGF2, and COMP cleared it in the GNPC alone.

In the GNPC dataset, several key protein alterations aligned with our initial LC–MS results for Alzheimer’s disease. Notably, the shared biomarkers ADIPOQ and IGFBP2 remained significantly elevated, consistent with the patterns observed in the LC–MS cohort. In addition, SHBG and IGF2 showed increased levels, while KLKB1 demonstrated a significant reduction, providing further support for both the shared and AD-specific protein signatures.

Moving to the Parkinson’s disease group, the results continued to show a consistent pattern. The shared marker IGFBP2 was significantly elevated, echoing the overlapping molecular signature identified between AD and PD in our discovery analysis. IGF2 also showed increased levels in PD, suggesting that this protein may represent a distinct PD-related feature captured in the GNPC cohort.

Lastly, we evaluated protein signatures associated with ALS. The GNPC analysis revealed a significant decrease in both COMP and CRTAC1 in ALS patients, consistent with the direction observed in our LC–MS dataset. The replication of these extracellular matrix–related proteins across independent proteomic

platforms suggests that matrix remodeling may represent a detectable molecular feature of motor neuron degeneration.

We also compared our findings with those reported in the GNPC analysis by Imam et al. (2025) (Supplementary Table 4). Six (SHBG, ADIPOQ, IGF2, IGFBP2, COMP, and CRTAC1) of the seven proteins that passed validation in our GNPC analysis showed consistent directional changes, whereas four proteins (EFEMP1, ITIH2, CFHR1, and APOC2) did not validate in our GNPC analysis but were significant in the Imam et al. report, with directions inconsistent with our LC-MS results. These differences may be attributable to variations in cohort definition. In our analysis, HCs were stringently defined as individuals free from neurodegenerative, metabolic, and inflammatory conditions, representing a strictly healthy population. In contrast, previous GNPC analyses defined controls primarily based on cognitive normality, which may introduce differences in baseline biological profiles.

We next asked whether the validated candidates depended on our strict, inflammation-free control definition. Each was retested in both cohorts against four control sets: HC, cognitively normal individuals without strong inflammation (CN[−SI]), cognitively normal individuals with strong inflammation (CN[+SI]), and individuals with strong inflammation but no neurodegenerative disease (SI) (Supplementary Table 5). Effect direction and nominal significance were largely preserved across the HC, CN(−SI), and CN(+SI) comparisons, so the signals do not arise from removing inflammatory participants. Against the SI group, KLKB1, CRTAC1, and PD-related IGFBP2 entries showed attenuated discriminative utility, suggesting that these markers may retain disease-associated relevance but are partly influenced by systemic inflammatory signals.

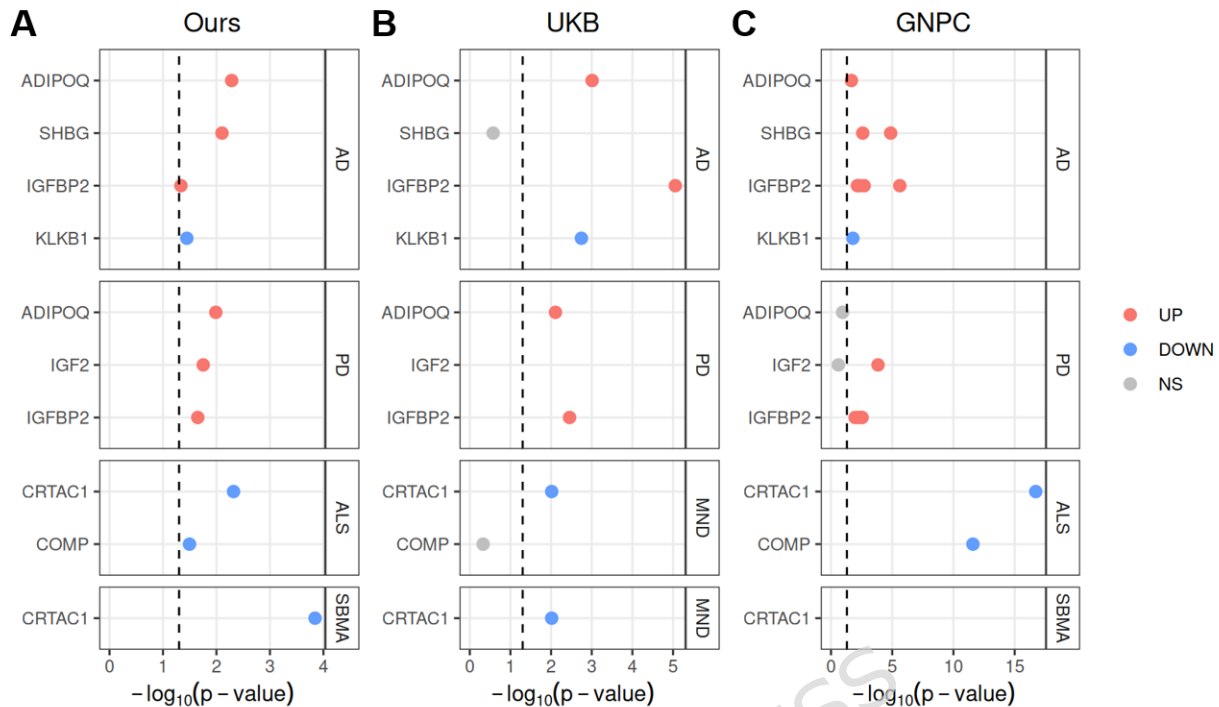


Figure 4. Cross-validation summary of candidate plasma protein signatures. Dot plots summarize the significance and direction of candidate protein changes in the LC–MS/MS discovery cohort (A), UK Biobank Olink data (B), and GNPC SomaScan data (C). Proteins are grouped by disease association. The x-axis shows $-\log_{10}(p\text{-value})$, and the dashed vertical line indicates nominal significance at $p < 0.05$. Red and blue dots indicate upregulated and downregulated proteins, respectively, while gray dots indicate non-significant associations. UKB motor neuron disease cases are shown as MND because ALS and SBMA could not be separated in that dataset.

To assess whether candidate discovery signals were dependent on the missing-value imputation strategy in our LC–MS/MS discovery, we repeated the differential expression analysis for the Figure 4 candidate proteins using four imputation schemes: left-shifted normal imputation (LSN), which was used in the primary analysis, deterministic imputation based on a minimal value approach (MinDet), probabilistic imputation based on a minimal value approach (MinProb), and quantile regression-based left-censored imputation (QRILC). For each imputed dataset, log2 fold changes and Mann–Whitney U test p-values were recalculated using the same disease-versus-control comparisons as in the primary discovery analysis. The resulting effect sizes and statistical significance values were summarized in Supplementary Figure 1.

Overall effect directions were largely preserved across imputation schemes. Statistical significance was likewise retained for most candidates, with the exception of IGFBP2, which fell below nominal significance ($p = 0.05$) under QRILC in the AD comparison and under both QRILC and MinProb in the PD comparison, and COMP, which lost significance under QRILC in the ALS comparison.

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Discussion

Our extensive plasma proteomics analysis has uncovered distinct protein signatures across major neurodegenerative disorders, highlighting both disease-specific and shared pathophysiological mechanisms. The identified protein candidates align with emerging literature on peripheral biomarkers in neurodegeneration and provide novel insights into disease-specific molecular pathways. Moreover, we validated our candidate proteins using two independent plasma proteomic datasets: the UKB Olink Explore 3072 dataset and the GNPC SomaScan dataset, which include ALS, AD, PD, and HC groups. Among the LC/MS-derived candidate proteins, 23 proteins were measurable in the UKB dataset and 20 proteins (represented by 27 SomaScan probes) were available in the GNPC dataset. In the UKB analysis, four proteins showed significant disease-associated differences compared with controls, and all four exhibited directional changes consistent with the LC–MS results. Similarly, in the GNPC dataset, seven proteins showed significant differences between disease groups and controls, and all seven displayed concordant directional changes with the LC–MS findings. In interpreting these results, we use the word reproducible here in a narrow way. A candidate earns that label only if its effect kept the same direction and remained significant on both platforms, Olink and SomaScan, and also held up when we changed how the control group was defined (Supplementary Table 5). Two proteins, IGFBP2 and CRTAC1, meet this bar. Others passed on a single platform, or faded once inflammatory participants were added to the comparison group, and for these we use the weaker description of directional concordance.

EFEMP1, an extracellular matrix glycoprotein, was identified as an AD-predominant protein in our discovery cohort, consistent with prior plasma proteomic findings [41]. However, this association was not consistently replicated across external validation datasets, suggesting that its relevance may be context-dependent or influenced by cohort-specific factors. As an extracellular matrix glycoprotein, EFEMP1 may still reflect processes related to neurovascular dysfunction, extracellular matrix remodeling, and parenchymal injury characteristic of AD pathophysiology, although further validation is required. In our AD cohort and the GNPC dataset, elevated plasma SHBG emerges as a potential biomarker for

distinguishing AD from other NDDs. However, this association was not consistently observed in the UK Biobank dataset, suggesting that its utility may be cohort-dependent. SHBG, another AD-predominant candidate in our cohort, was significantly elevated in the GNPC validation dataset. While less frequently reported in prior plasma proteomics studies, these findings align with SHBG's established role as a sex hormone carrier that regulates the bioavailability of neuroprotective steroids and influences brain aging through endocrine pathways [42, 43]. Notably, higher plasma SHBG levels predicted accelerated neurodegeneration, including CSF A β 42 decline, hippocampal atrophy, and cognitive decline in prodromal AD, suggesting its potential as a prognostic marker for AD progression [42].

Decreased plasma KLKB1 levels were consistently observed across our AD cohort, the UK Biobank cohort, and the GNPC dataset, suggesting a reproducible signal across independent platforms. Although direct plasma proteomic associations for KLKB1 in AD remain limited, the kallikrein-kinin system has been implicated in AD pathophysiology, suggesting that peripheral KLKB1 downregulation may reflect altered system dynamics or CNS sequestration [44]. The kallikrein-kinin system modulates blood-brain barrier permeability, cerebrovascular tone, and transendothelial leukocyte trafficking, potentially contributing to AD pathophysiology through enhanced neuroinflammation and vascular dysfunction [45, 46]. The consistent reduction of KLKB1 observed across both the UKB and GNPC cohorts further supports the potential involvement of coagulation–inflammatory pathways in AD-related pathophysiology.

Beyond coagulation-related pathways, metabolic markers also yielded consistent signals across platforms. Plasma ADIPOQ was upregulated in AD in our discovery cohort, the UKB, and the GNPC, supporting a stable association between this adipokine and AD pathophysiology. This observation aligns with multiple prior studies reporting elevated adiponectin levels in neurodegenerative conditions, including AD and PD [47–49]. In contrast, while ADIPOQ levels were also increased in PD in our LC–MS/MS and UKB analyses, this trend was not consistently observed in the GNPC dataset, suggesting that its association with PD may be less robust or context-dependent. Adiponectin functions as a critical metabolic and inflammatory mediator through its receptors AdipoR1 and AdipoR2, regulating insulin sensitivity, AMPK signaling, and

immune responses [50]. Systemic metabolic dysfunction—including insulin resistance, impaired glucose homeostasis, and chronic low-grade inflammation—represents a convergent pathogenic pathway across AD, MCI, and PD. These metabolic alterations may be mediated by disrupted adiponectin signaling, enhanced neuroinflammation, and impaired mitochondrial function [47, 51, 52].

Trophic support mechanisms involving growth factor-related proteins—particularly IGF2 in PD and IGFBP2 across multiple neurodegenerative conditions—represent critical disease pathways. IGF2 exerts neuroprotection through multiple mechanisms: preventing dopaminergic neuronal loss, reducing oxidative stress, and decreasing intracellular alpha-synuclein accumulation [53-55]. Beyond direct cytoprotection, IGF2 modulates neuroinflammatory responses, addressing both primary neurodegeneration and secondary neuroinflammation [56, 57]. These dual mechanisms position IGF2 as a compelling therapeutic target for PD. In our analysis, IGF2 showed increased levels and passed validation in both LC-MS/MS and GNPC datasets, supporting its relevance in PD; however, it could not be evaluated in the UK Biobank cohort due to the absence of a corresponding target in the Olink Explore 3072 panel. IGFBP2 similarly shows associations with Alzheimer's disease biomarkers and cognitive decline across neurodegenerative diseases, suggesting that impaired growth factor signaling represents a shared pathobiological mechanism amenable to therapeutic intervention [58, 59]. Notably, IGFBP2 consistently passed validation in both UKB and GNPC, with particularly strong support in GNPC where multiple SOMAmer measurements independently confirmed its signal. The observation that both ADIPOQ and IGFBP2 are consistently elevated in AD and PD across our discovery cohort and the two external validation datasets reinforce the notion that these alterations reflect shared disease mechanisms. This multi-cohort consistency suggests that metabolic and trophic signaling alterations are likely shared, underlying features of these conditions, rather than isolated findings.

The convergent downregulation of CRTAC1 and COMP in both ALS and SBMA indicates shared pathological mechanisms distinguishing motor neuron diseases from other neurodegenerative conditions. These proteins' roles in coagulation, cartilage homeostasis, and ECM organization reflect motor neurons'

unique susceptibility to metabolic stress and structural dysfunction [60, 61]. The dysregulation of ECM-related proteins may compromise structural integrity and trophic support essential for motor neuron viability under chronic metabolic stress [62, 63]. Disrupted ECM homeostasis and coagulation-related pathways may represent distinctive features of motor neuron degeneration [60, 64, 65]. The robust replication of decreased CRTAC1 and COMP levels in our external validation analyses supports this concept. CRTAC1 was consistently validated across both UKB and GNPC, whereas COMP showed replication only in GNPC. Although both proteins display concordant downregulation, further validation is needed to confirm the robustness of COMP. COMP is a notable case. It fell in the expected direction in the UK Biobank but did not reach significance (Std. Coef. = 0.052, $p = 0.469$; Supplementary Table 3), so for that cohort we treat it as directionally concordant, not validated.

Two complementary interpretations may explain the shared downregulation of these proteins. One is a shared neurodegenerative mechanism. The other is shared downstream turnover of muscle and connective tissue, a reading that fits SBMA particularly well, since SBMA, unlike ALS, is now treated as a neuromuscular disorder with a primary, cell-autonomous muscle component [36-38]. Both proteins are connective-tissue glycoproteins [40], so their circulating levels may track muscle and connective-tissue pathology as much as neuronal loss. Plasma data on their own cannot settle which source dominates.

This uncertainty about tissue of origin highlights a broader challenge in interpreting circulating biomarkers. Several arise mainly in the liver, in metabolic tissue, or in connective tissue rather than in neurons, so a plasma value blends signals from more than one organ. That feature cuts both ways. The peripheral metabolic and trophic axis traced by ADIPOQ, IGFBP2, and IGF2 may itself prove useful for following disease over time, and plasma has the practical advantages of being minimally invasive and easy to resample. Yet because the proteins come from many sources, reading any single marker as disease-specific is risky, and these candidates are better suited to screening and longitudinal monitoring than to diagnosis on their own.

Beyond our discovery findings, recent advances in targeted proteomics have further expanded the biomarker landscape in motor neuron diseases. The NULISA (Nucleic-acid-Linked Immuno-Sandwich Assay) platform, a high-sensitivity multiplexed immunoassay, has shown particular utility for identifying and monitoring neurodegeneration-associated proteins in ALS and related conditions [66-68]. Studies using NULISA have confirmed elevated neurofilament light and heavy chains, phospho-tau species (pTau181, pTau217, pTau231), and phosphorylated TDP-43 in ALS plasma, with phospho-tau levels inversely correlating with disease progression and survival [67]. NULISA has also proven valuable for tracking treatment-responsive biomarkers, including neurofilament proteins, amyloid- β peptides, and neuropentraxin family members, in SOD1-ALS patients receiving tofersen, underscoring its potential as an endpoint tool in clinical trials [66]. These targeted approaches complement our unbiased LC-MS/MS discovery strategy. Whereas our workflow identified novel motor neuron disease-enriched candidates such as CRTAC1 and COMP, along with cross-disease signatures including IGFBP2 and ADIPOQ, NULISA-based platforms enable sensitive, high-throughput quantification of pre-defined neurodegeneration-relevant proteins. Integrating both strategies may therefore provide the most comprehensive framework for biomarker characterization in motor neuron diseases.

Several limitations should be considered when interpreting these findings. First, the cross-sectional design precludes causal inference and does not permit evaluation of temporal relationships between proteomic alterations and disease progression. Longitudinal sampling will be required to determine whether the observed changes precede, accompany, or follow clinical onset.

Second, the relatively small sample sizes in our discovery cohort, although typical of LC-MS/MS-based proteomic studies at the discovery stage, impose constraints on statistical power. Accordingly, we applied nominal significance thresholds without multiple-comparison correction, and demographic covariates such as age and sex were not included in the primary analysis to preserve the limited degrees of freedom. To compensate for this, covariate-adjusted models were employed in the substantially larger UKB and GNPC cohorts, where adequate sample sizes permitted more comprehensive control for potential confounders.

Third, inherent run-to-run variability in LC–MS/MS acquisition may introduce measurement noise that complicates cross-sample comparisons. We sought to mitigate this concern by replicating key findings in the UKB and GNPC datasets, both of which were generated on orthogonal proteomic platforms. Although several candidate proteins exhibited concordant directions of change across all three datasets, incomplete overlap in platform-specific protein coverage—including the absence of certain targets from the Olink Explore 3072 or SomaScan panels—limits full cross-platform alignment and may partly explain discrepancies for individual markers.

A further limitation concerns the source material. Because every participant was a living patient, post-mortem brain tissue was unavailable, and CSF, though obtainable in living individuals, was not part of the sampling protocol used here. Plasma therefore offers only an indirect read on CNS pathology, and the candidate signals cannot be tied firmly to the brain on the basis of blood alone. Confirming these markers in CSF, which would be feasible in a prospective extension of this cohort, is an important next step.

We were also unable to test whether the motor-neuron candidates behave the same way in primary myopathies or muscular dystrophies. The UK Biobank places SBMA and related conditions under a single motor neuron disease code (ICD-10 G12.2), so primary muscle-disease cases cannot be pulled out separately, and the GNPC carries no clinical annotation for SBMA or muscular dystrophy. Answering this will require cohorts built specifically around muscle disease.

Finally, SBMA is an X-linked disorder affecting only males, and in our cohort the SBMA group was entirely male and younger than the controls. Although this reflects the underlying biology, the discovery analysis did not adjust for age or sex, limiting the ability to disentangle SBMA-associated signals from potential demographic confounding, particularly given established sex-related differences in the circulating proteome. In addition, the absence of SBMA cases in the external datasets precluded independent validation of these findings. Therefore, the SBMA-predominant signals should be interpreted with caution and regarded as hypothesis-generating. Future studies incorporating sex-stratified analyses and appropriately age- and sex-matched control cohorts will be important to clarify disease-specific effects.

Taken together, validation in larger, longitudinal, and technically harmonized cohorts will be essential to confirm the robustness of the biomarker signatures reported here and to establish their clinical utility.

Conclusions

This comprehensive plasma proteomics analysis identified distinct molecular signatures across major neurodegenerative disorders, providing valuable insights into both disease-specific and shared pathophysiological mechanisms. These findings highlight the potential of peripheral blood biomarkers to advance our understanding of neurodegeneration and offer promising avenues for diagnostic and therapeutic development. However, rigorous validation studies and mechanistic investigations are essential to translate these discoveries into clinical applications that benefit patients with neurodegenerative diseases.

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List of abbreviations

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Neurodegenerative diseases (NDDs)

Spinal and bulbar muscular atrophy (SBMA)

Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

Alzheimer's disease (AD)

Parkinson's disease (PD)

Amyotrophic lateral sclerosis (ALS)

Central nervous system (CNS)

Peripheral nervous system (PNS)

Global Neurodegeneration Proteomics Consortium (GNPC)

UK Biobank (UKB)

Healthy controls (HCs)

Mild cognitive impairment with amyloid PET positivity (ADMCI)

Amyloid-negative mild cognitive impairment (MCI)

Korean mini-mental state examination (K-MMSE)

Clinical dementia rating (CDR)

CDR sum of boxes (CDR-SB)

Bayer activities of daily living (B-ADL)

Korean instrumental activities of daily living (K-IADL)

Unified Parkinson's disease rating scale (UPDRS)

Schwab and England activities of daily living (SEADL)

Amyotrophic lateral sclerosis functional rating scale (ALSFRS-R)

6-minute walk test (6MWT)

Dementia with early AD pathology (BAPL2)

Motor neuron disease (MND)

Vascular dementia (VaD)

Scans without evidence of dopaminergic deficit (SWEDD)

Normal pressure hydrocephalus (NPH)

AD with comorbid NPH (AD/cNPH)

Atypical parkinsonism with comorbid NPH (APD/cNPH)

Hereditary spastic paraplegia (HSP)

Sporadic adult-onset ataxia (SAOA)

Dementia with Lewy bodies (DLB)

Frontotemporal lobar degeneration (FTLD)

Vascular parkinsonism (VaP)

Molecular weight cut-off (MWCO)

Ammonium bicarbonate (ABC)

Bicinchoninic acid (BCA)

Dithiothreitol (DTT)

Iodoacetamide (IAA)

Label-free quantification (LFQ)

Match between runs (MBR)

False discovery rate (FDR)

Differentially expressed proteins (DEPs)

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The GNPC data in the preparation of this article were obtained on Sep. 19, 2025 from the Global Neurodegeneration Proteomics Consortium (GNPC) Harmonized Data Superset Version 1 (<https://www.neuroproteome.org/v1-harmonized-data-set-hds>). While the investigators within the Global Neurodegeneration Proteomics Consortium contributed data to the Global Neurodegeneration Proteomics Consortium, they did not necessarily participate in the analysis or writing of this report.

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Author contributions

All authors contributed to the article and approved the submitted version.

H.-J.K., and M.C. contributed to the conception and design of the study.

S.L., and H.-J.K. contributed to clinical sample acquisition and experimental analysis.

H.-W.L., and J.-S.P. contributed to clinical sample acquisition and phenotyping.

E.C., and J.H.Y. contributed to LC–MS/MS experiments.

A.J., and W.Y. contributed to LC–MS/MS data processing and analysis.

Y.C., and M.C. contributed to computational and statistical analysis, including UK Biobank and Global Neurodegeneration Proteomics Consortium datasets.

Y.C., S.L., H.-J.K., and M.C. contributed to data interpretation and drafting substantial portions of the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All participants underwent clinical evaluation at the Neurodegenerative Disease Center of Kyungpook National University Chilgok Hospital (KNUCH) during the period from 2020 to 2023. Written informed consent was obtained from each participant and, where applicable, from their legally authorized caregivers prior to enrollment. The study protocol was reviewed and approved by the Institutional Review Board of KNUCH (approval number: KNUMC-2023-01-036-001).

Consent for publication

Not applicable.

Competing interests

All authors declare no conflict of interest that might potentially bias this work.

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Table 1. Demographic and Clinical Characteristics of Study Participants

	HC (N=58)		AD (N=34)		PD (N=30)		MCI (N=20)		ADMCI (N=20)		ALS (N=20)		SBMA (N=42)	
	NA (%)	mean $\pm$ SD	NA (%)	mean $\pm$ SD	NA (%)	mean $\pm$ SD	NA (%)	mean $\pm$ SD	NA (%)	mean $\pm$ SD	NA (%)	mean $\pm$ SD	NA (%)	mean $\pm$ SD
Age (year)	-	67.4 $\pm$ 7.95	-	75.5 $\pm$ 7.31	-	72.6 $\pm$ 7.59	-	74.9 $\pm$ 6.70	-	74.2 $\pm$ 7.63	-	63.0 $\pm$ 9.36	-	57.4 $\pm$ 11.1
Sex (F:M)	-	42:16 (72.4: 27.6)	-	26:8 (76.5: 23.5)	-	13:17 (43.3: 56.7)	-	15:5 (75.0: 25.0)	-	13:7 (65.0: 35.0)	-	6:14 (30.0: 70.0)	-	0:42 (0:10 0)
Age at symptom onset (year)	-	-	-	70.3 $\pm$ 8.07	-	64.2 $\pm$ 7.51	2 (10.0)	68.6 $\pm$ 8.20	-	69.4 $\pm$ 6.97	-	60.6 $\pm$ 10.1	-	45.9 $\pm$ 11.7
Disease duration (year)	-	-	-	5.14 $\pm$ 2.88	-	8.43 $\pm$ 3.53	2 (10.0)	6.50 $\pm$ 3.88	-	4.85 $\pm$ 4.13	-	2.60 $\pm$ 2.80	-	11.9 $\pm$ 8.66
K-MM SE score	17 (29.3)	28.5 $\pm$ 1.52	-	15.3 $\pm$ 7.47	2 (6.67)	24.75 $\pm$ 4.12	-	24.7 $\pm$ 2.83	-	25.6 $\pm$ 3.15	-	-	-	-
CDR	17 (29.3)	0.29 $\pm$ 0.25	-	1.41 $\pm$ 0.89	2 (6.67)	0.41 $\pm$ 0.27	-	0.50 $\pm$ 0.00	-	0.50 $\pm$ 0.00	-	-	-	-

CDR-SB	17 (29.3)	0.35 ±0.32	-	8.19 ±4.84	2 (6.67)	1.68 ±1.94	-	1.50 ±0.63	-	1.93 ±0.95	-	-	-	-
GDS	17 (29.3)	1.95 ±0.59	1 (2.94)	4.82 ±0.95	2 (6.67)	2.54 ±1.00	-	2.90 ±0.31	-	3.00 ±0.46	-	-	-	-
B-ADL	17 (29.3)	19.5 ±3.12	-	17.9 ±4.04	2 (6.67)	18.5 ±2.82	-	19.95 ±0.22	-	19.95 ±0.22	-	-	-	-
K-IADL	17 (29.3)	0.05 ±0.15	-	1.51 ±0.91	2 (6.67)	0.30 ±0.41	-	0.21 ±0.18	-	0.23 ±0.13	-	-	-	-
UPDRS Part I	-	-	-	-	1 (3.33)	3.69 ±2.63	-	-	-	-	-	-	-	-
UPDRS Part II	-	-	-	-	1 (3.33)	11.7 ±7.34	-	-	-	-	-	-	-	-
UPDRS Part III	-	-	-	-	-	22.7 ±8.79	-	-	-	-	-	-	-	-
UPDRS Part IV	-	-	-	-	1 (3.33)	1.38 ±1.93	-	-	-	-	-	-	-	-
Modified H&Y	-	-	-	-	-	2.33 ±0.38	-	-	-	-	-	-	-	-
SEADL	-	-	-	-	1 (3.33)	81.4 ±16.0	-	-	-	-	-	-	-	-
ALSFRS-R	-	-	-	-	-	-	-	-	-	-	3 (15.0)	30.2 ±10.8	1 (2.38)	37.9 ±4.62
6MWT	-	-	-	-	-	-	-	-	-	-	20 (10.0)	-	4 (9.52)	290 ±139

Note: Data are presented as mean±SD or n(%).

Abbreviations: AD, Alzheimer's disease; ADMCI, amyloid-positive mild cognitive impairment; ALS, amyotrophic lateral sclerosis; ALSFRS-R, amyotrophic lateral sclerosis functional rating scale-revised; B-ADL, Bayer activities of daily living; CDR, clinical dementia rating; CDR-SB, CDR sum of boxes; GDS, global deterioration scale; HC, healthy control; K-IADL, Korean instrumental activities of daily living; K-MMSE, Korean mini-mental state examination; MCI, mild cognitive impairment; Modified H&Y, modified Hoehn and Yahr stage; NA, not available or not applicable (missing data); PD, Parkinson's disease; SBMA, spinal and bulbar muscular atrophy; SEADL, Schwab and England activities of daily living; UPDRS, unified Parkinson's disease rating scale; UPDRS part I, behavior and emotion; UPDRS part II, activities of daily living; UPDRS part III, motor scale; UPDRS part IV, drug complications; 6MWT, 6-minute walk test.

Table 2. Key protein signatures derived from the cross-disease comparison of neurodegenerative disorders

AD predominant	
Up	CDH13, EFEMP1, SHBG
Down	ITIH1, ITIH2, KLKB1
PD predominant	
Up	CFHR1, IGF2, LYZ
Down	-
AD & PD in common	
Up	ADIPOQ, IGFBP2
Down	APOC2
ALS predominant	
Up	C4BPB, CDH13
Down	CALR, CPB2, CSF1R, EFEMP1
SBMA predominant	
Up	AFM, APOC4, C3, CPN2, FCN3, FETUB
Down	APOD, C9, CA1, CD44, CD5L, CFHR5, F10, FBLN1, FCGBP, HPR, IGFBP2, NRP1
ALS & SBMA in common	
Up	-
Down	COMP, CRTAC1, F13B

Categorization is based on a significance threshold of $p < 0.05$, identifying proteins as either up- or down-regulated

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; PD, Parkinson's disease; SBMA, spinal and bulbar muscular atrophy; Std. Coef., standardized coefficient.

Table 3. Validation of candidate proteins using the Olink-based UKB cohort

UKB Disease	UniProt ID	Gene Symbol	UKB		LC-MS Trend (predominance)
			Trend	Std. Coef. (p-value)	
AD	P03952	KLKB1	Down	-0.132 (0.002)	Down (AD)
	Q15848	ADIPOQ	Up	0.130 (0.001)	Up (AD & PD)
	P18065	IGFBP2	Up	0.182 (<0.001)	Up (AD & PD)
PD	Q15848	ADIPOQ	Up	0.121 (0.008)	Up (AD & PD)
	P18065	IGFBP2	Up	0.109 (0.004)	Up (AD & PD)
MND	Q9NQ79	CRTAC1	Down	-0.181 (0.010)	Down (ALS & SBMA)

Only proteins with a p-value < 0.05 in the UKB are included in the table.

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; MND, motor neuron disease; PD, Parkinson's disease; SBMA, spinal and bulbar muscular atrophy; Std. Coef., standardized coefficient; UKB, UK biobank.

Table 4. Validation of candidate proteins using the SomaLogic-based GNPC dataset

GNPC Disease	SomaLogic Seq ID	Gene Symbol	GNPC		LC-MS Trend (predominance)
			Trend	Std. Coef. (p-value)	
AD	4929-55	SHBG	Up	0.092 (0.002)	Up (AD)
	7909-37	SHBG	Up	0.033 (<0.001)	Up (AD)
	4152-58	KLKB1	Down	-0.061 (0.005)	Down (AD)
	3554-24	ADIPOQ	Up	0.067 (0.021)	Up (AD & PD)
	22985-160	IGFBP2	Up	0.136 (<0.001)	Up (AD & PD)
	2570-72	IGFBP2	Up	0.084 (0.005)	Up (AD & PD)
	8469-41	IGFBP2	Up	0.050 (<0.001)	Up (AD & PD)
	8819-3	IGFBP2	Up	0.092 (0.002)	Up (AD & PD)
PD	15295-81	IGF2	Up	0.262 (<0.001)	Up (PD)
	22985-160	IGFBP2	Up	0.176 (0.003)	Up (AD & PD)
	2570-72	IGFBP2	Up	0.156 (0.011)	Up (AD & PD)
	8469-41	IGFBP2	Up	0.170 (0.006)	Up (AD & PD)
	8819-3	IGFBP2	Up	0.177 (0.004)	Up (AD & PD)
ALS	8043-153	COMP	Down	-0.931 (<0.001)	Down (ALS & SBMA)
	5632-6	CRTAC1	Down	-1.031 (<0.001)	Down (ALS & SBMA)

Only proteins with a p-value < 0.05 in the GNPC are included in the table.

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; GNPC, global neurodegeneration proteomics consortium; PD, Parkinson's disease; SBMA, spinal and bulbar muscular atrophy; Std. Coef., standardized coefficient.