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Associations of plasma SMOC1 and soluble IL6RA levels with the progression from mild cognitive impairment to dementia

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ABSTRACT

Despite the central role attributed to neuroinflammation in the etiology and pathobiology of Alzheimer's disease (AD), the direct link between levels of inflammatory mediators in blood and cerebrospinal fluid (CSF) compartments, as well as their potential implications for AD diagnosis and progression, remains inconclusive. Moreover, there is debate on whether inflammation has a protective or detrimental effect on disease onset and progression. Indeed, distinct immunological mechanisms may govern protective and damaging effects at early and late stages, respectively.

This study aims to (i) identify inflammatory mediators demonstrating robust correlations between peripheral and central nervous system (CNS) compartments by means of plasma and CSF analysis, respectively, and (ii) assess their potential significance in the context of AD and disease progression from mild cognitive impairment (MCI) to dementia. To achieve this, we have examined the inflammatory profile of a well-defined subcohort comprising 485 individuals from the Ace Alzheimer Center Barcelona (ACE). Employing a hierarchical clustering approach, we thoroughly evaluated the intercompartmental correlations of 63 distinct inflammation mediators, quantified in paired CSF and plasma samples, using advanced SOMAscan technology. Of the array of mediators investigated, only six mediators (CRP, IL1RAP, ILRL1, IL6RA, PDGFRB, and YKL-40) exhibited robust correlations between the central and peripheral compartments (proximity scores <400). To strengthen the validity of our findings, these identified mediators were subsequently validated in a second subcohort of individuals from ACE (n = 873). The observed plasma correlations across the entire cohort consistently have a Spearman rho value above 0.51 (n = 1,360, p < 1.77E-93).

Of the high CSF-plasma correlated proteins, only soluble IL6RA (sIL6RA) displayed a statistically significant association with the conversion from MCI to dementia. This association remained robust even after applying a stringent Bonferroni correction (Cox proportional hazard ratio [HR] = 1.936 per standard deviation; p = 0.0018). This association retained its significance when accounting for various factors, including CSF amyloid (Aβ42) and Thr181-phosphorylated tau (p-tau) levels, age, sex, baseline Mini-Mental State Examination (MMSE) score, and potential sampling biases identified through principal component analysis (PCA) modeling.

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Furthermore, our study confirmed the association of both plasma and CSF levels of SPARC-related modular calcium-binding protein 1 (SMOC1) with amyloid and tau accumulation, indicating their role as early surrogate biomarkers for AD pathology. Despite the lack of a statistically significant correlation between SMOC1 levels in CSF and plasma, both acted as independent biomarkers of disease progression (HR > 1.3, $p < 0.002$).

In conclusion, our study unveils that sIL6RA and SMOC1 are associated with MCI progression. The absence of correlations among inflammatory mediators between the central and peripheral compartments appears to be a common pattern, with only a few intriguing exceptions.

1. Introduction

Alzheimer's disease (AD) stands as the prevailing cause of dementia in elderly adults, making it a substantial healthcare concern. Currently, this disease is estimated to affect 50 million people worldwide, and this number is projected to increase to 150 million by 2050, according to the most recent data (Juul Rasmussen and Frikke-Schmidt, 2023). From a clinical perspective, AD follows a progressive course characterized by different stages (Jack et al., 2024). AD starts with a long asymptomatic phase, known as the preclinical stage, in which pathological changes accumulate in the brain without noticeable clinical symptoms. Subsequently, individuals may enter a prodromal stage marked by mild cognitive alterations, observed as deficits in formal neuropsychological tests, particularly affecting memory and executive functions. This stage is commonly referred to as mild cognitive impairment (MCI). As the disease advances, individuals progress to the final stage of overt dementia, experiencing progressive loss of independence in performing daily tasks. This decline eventually leads to complete dependence due to the inability to carry out basic activities.

AD is characterized by distinctive pathological features in the brain, including the deposition of amyloid in the interstitial space of brain tissue (amyloid plaques) and in blood vessels (cerebral amyloid angiopathy), the formation of tau deposits as intraneuronal fibrillary tangles (NFTs), pronounced brain inflammation marked by gliosis, and neuronal cell loss. Moreover, it is increasingly recognized that AD often coexists with additional comorbid pathologies in the brain. Rather than being the exception, these brain co-pathologies are commonly observed and may potentially synergize to accelerate disease progression (Tomé and Thal, 2021).

While the precise cause of AD remains incompletely understood, it is widely acknowledged that inflammation plays a key role in the disease. The inflammatory hypothesis of AD suggests that chronic brain inflammation may significantly contribute to the development and progression of the disease (McGeer and McGeer, 2013; Walker et al., 2023; Tripathi et al., 2024; Bellenguez et al., 2022a). Genome-wide association studies (GWAS)(Sims et al., 2017; Kunkle et al., 2019) and pathway analyses (Jones et al., 2015) have uncovered a multitude of loci that influence AD risk, with a notable enrichment of genes expressed in microglia, the brain's resident macrophages (Helmut et al., 2011). Notably, AD-associated genetic variants are frequently found in regions linked to key immunological functions, such as the human leukocyte antigen (HLA) complex (Le Guen et al., 2023), the triggering receptor expressed on myeloid cells 2 (TREM2) signaling cascade (*TREM2, PLCG2, IL34, CD33*) (Sims et al., 2017; Jonsson et al., 2013; de Rojas et al., 2021), and the linear ubiquitin chain assembly (LUBAC) complex, which plays a critical role in signaling pathways that modulate the activation of nuclear factor kappa B (NF-κB), a well-established inflammatory transcription factor (Bellenguez et al., 2022b).

Here we present our efforts (i) to identify inflammatory mediators with robust correlations between the plasma and cerebrospinal fluid (CSF) compartments and (ii) to assess their potential significance in the context of AD and the progression from MCI to dementia. To achieve this, we leveraged paired matched plasma-CSF SOMAscan proteome data of a well-defined cohort from the Ace Alzheimer Center Barcelona (ACE). We extracted selected inflammatory molecules and analyzed them using a hierarchical clustering approach, and we thoroughly evaluated the intercompartmental correlations of distinct inflammation mediators and their relationships with hallmarks of AD pathology in the context of the AD continuum.

2. Material & methods

2.1. Standard protocol approvals, registrations, and patient consent

All the study's sample collection protocols have been approved by the Clinical Research Ethics Commission of the Hospital Clinic (Barcelona, Spain) in accordance with the Declaration of Helsinki and the current Spanish regulations in the field of biomedical research. Likewise, in accordance with Spain's Data Protection Law, all participants were informed about the study's goals and procedures by a neurologist before signing an informed consent form. Patients' privacy and data confidentiality were protected in accordance with applicable laws.

1. The ACE CSF cohort and sample acquisition

Since 2016, ACE has been building a CSF cohort (the ACE CSF cohort). It is a real-world database comprising consecutive individuals who have sought cognitive assessment at ACE's Memory Clinic and who were considered eligible for lumbar puncture (LP) for diagnostic purposes.

This cohort encompasses a comprehensive collection of baseline samples, gathered on the day of the LP, including matched CSF, plasma, serum, saliva, and peripheral blood mononuclear cell (PBMC) specimens. All biospecimens obtained were part of the ACE collection, registered in Instituto de Salud Carlos III (ISCIII, Ministry of Health of Spain) with the code C.0000299. These biological samples were meticulously paired with contemporary records, including demographic, cognitive, and neurological information, along with brain MRI data. Notably, the window for acquiring samples and the associated clinical record was less than three months for all patients. The cohort maintains regular follow-ups with the patients, acquiring neurological and neuropsychological data on an annual basis. For an in-depth understanding of the methodology, additional information can be found in a recent publication (Orellana et al., 2022).

Briefly, the ACE CSF cohort includes patients with MCI and dementia from ACE's Memory Clinic, research participants with subjective cognitive decline (SCD) from the Fundació ACE Healthy Brain Initiative (FACEHBI)(Rodriguez-Gomez et al., 2017), and patients with early-onset MCI from the BIOFACE study (Esteban De Antonio et al., 2021). The study participants were patients who sought consultation for cognitive impairment and were evaluated at the memory clinic of the Ace Alzheimer Center in Barcelona, Spain. Each patient received a consensus diagnosis from a multidisciplinary team, including neurologists, neuropsychologists, and social workers. All participants underwent assessments using the Spanish version of the Mini-Mental State Examination (MMSE), the memory component of the Spanish version of the 7 Minute Test, the Spanish version of the Neuropsychiatric Inventory Questionnaire (NPI-Q) (34), the Hachinski Ischemia Scale, the Blessed Dementia Scale, the Clinical Dementia Rating (CDR) scale, and a comprehensive neuropsychological battery developed by Ace (N-BACE) as described previously (Marquié et al., 2023). Subjects who converted to dementia, including AD, vascular dementia, mixed dementia (AD

with cerebrovascular disease), frontotemporal dementia, or dementia with Lewy bodies over the study period, were classified as MCI converters according to our previous definitions (Espinosa et al., 2013). All of these subjects had a Clinical Dementia Rating (CDR) of 1. In contrast, those subjects who remained stable during follow-ups were classified as stable or non-MCI converters.

CSF acquisition and analysis procedures follow consensus recommendations (Hansson et al., 2021). Blood samples were collected in polypropylene tubes with K2-EDTA (BD Vacutainer). CSF samples were collected in polypropylene tubes (Sarstedt Ref 62.610.018). Plasma was separated by centrifugation (2000×*g*, 10 min, 4 °C), as well as CSF for common AD biomarker determination. Then, both specimens were aliquoted and stored in polypropylene tubes (Sarstedt Ref 72.694.007) at −80 °C until use.

The CSF Aβ40, Aβ42, t-tau, and p-tau181 biomarkers were quantified by using the Lumipulse G600II automated platform (Fujirebio Europe, Göteborg, Sweden) or Innotest® ELISA immunoassays (Fujirebio Europe, Göteborg, Sweden), with quality control measures in place. Cutoffs for AT(N) classification and the standardization of Aβ42 results for comparability across different platforms have also been established (Orellana et al., 2022).

2. Protein analysis in CSF and plasma using the SOMAscan panel and protein selection for analysis

A subset of 1369 paired plasma and CSF samples underwent extensive analyses using the SOMAscan 7k panel, a high-throughput proteomic assay provided by SomaLogic Operating Co., Inc., based in Boulder, Colorado. This advanced assay was designed to simultaneously measure the abundance of more than 7000 proteins in both plasma and CSF samples. Briefly, the procedure involves the use of 50 μL of either CSF or plasma, employing modified DNA aptamers, also called somamers, to quantify protein levels. Initially, proteins were captured by these immobilized aptamers, facilitated by streptavidin beads, and subsequently tagged with fluorescent markers. Following the elimination of unbound proteins, the streptavidin beads were released using ultraviolet light. The resulting protein-aptamer complexes were re-captured by monomeric avidin, as described previously (Gold et al., 2010). Finally, the measurements of protein levels, expressed in relative fluorescent units (RFUs), were subjected to normalization using the adaptive normalization by maximum likelihood method, as elucidated previously (Candia et al., 2017). Data generated will be available at the Global Neurodegeneration Proteomics Consortium via the Alzheimer Disease Data Initiative (ADDI) portal (https://www.neuroproteome.org/), or by direct request to the ACE Alzheimer Center Barcelona.

2.1.1. Selection of inflammatory-related analytes and quality scoring of the selected protein expression measurements

When Coefficient of variation (CV) was compared between SOMAscan and Olink measurements, SOMAscan was more uniform in both intra- and interassay CV evaluations compared to the Olink® Explore panels. For this reason, from the comprehensive SOMAscan panel, we selected 85 different somamers associated with inflammation. This selection was based on their presence in the previous literature (Gigase et al., 2023; Sun et al., 2003; Whelan et al., 2019a), data availability on proteomic platforms (SOMAscan and Olink Explore), and categorization through inter- and intra-platform comparison. The selection of 85 somamers targeting 63 immunological mediators was designed to cover a broad range of inflammatory pathways including interleukins, chemokines, complement proteins, acute phase proteins, and growth factors. Overlap in sequences was included to have a detailed analysis of specific domains or variants of key inflammatory mediators, providing a comprehensive view of the inflammatory response in CSF and plasma samples. This approach enabled us to explore systemic inflammation and potential correlations with cerebrospinal fluid markers in various disease contexts. The variation in the number of somamers and proteins is due to the existence of multiple somamers and Olink measures for certain selected proteins. The list includes well-established proteins such as CRP, YKL-40, GFAP, and TREM2, along with various interleukins and their receptors, complement cascade factors, microglial migration stimulants, and other trophic signals (see Supplementary Table 1). Previous findings showed disparities in the expression levels of multiple analytes measured using the SOMAscan platform, both within and between platforms (Puerta et al., 2023). In light of these findings, we opted to categorize selected features based on our recent classification, considering the consistency observed in both intra- and inter-platform comparisons. This involved evaluating (i) correlations between aptamers measured in two independent studies conducted on the same samples using the SOMAscan platform and (ii) correlations between SOMAscan and Olink proteomics (Puerta et al., 2023). In line with this classification, the markers selected for this study configured 94 intra-platform and/or inter-platform pairs that were categorized as follows: 56 pairs demonstrated a good level of reproducibility (including six without Olink-SOMAscan inter-platform data; Spearman rho $\geq$0.5), 15 pairs displayed moderate performance (two without inter-platform data; 0.5 $<$ Spearman rho $\geq$0.3), and 23 pairs showed poor performance (four without inter-platform data; Spearman rho $<$0.3). Proteins with both intra- and interassay moderate –to-good correlation were prioritized when possible. Detailed and aggregated quality control results can be found in Supplementary Tables 1 and 2, respectively.

3. Statistical methods

a. Identification of proteins associated with dementia

To investigate the disease continuum from cognitively healthy to MCI and dementia stages, analysis of variance (ANOVA) tests were performed to compare the mean values of each somamer. Additionally, Spearman's rho coefficients were calculated for all paired plasma-CSF values of each somamer, facilitating an examination of the relationships between CSF and plasma protein levels. This comprehensive analysis was carried out using the Statistical Package for the Social Sciences (SPSS) 26.0 (IBM Corp., Armonk, NY, USA). To control for multiple comparisons, a Bonferroni-corrected study-wide significance level for the ANOVA test was set at p = 5.88E-4, considering a total of 85 comparisons. In parallel, significant proteins were further assessed as potential mediators of disease progression through Cox proportional hazard risk models (see below).

b. Principal component analysis (PCA) and source of bias detection in SOMAscan proteomic results

To explore the potential impact of bias and confounding factors on SOMAscan results, principal component analysis (PCA) was carried out using data exclusively from selected somamers. In a nutshell, the SOMAscan results for each protein were subjected to a log10 transformation, outliers beyond ±3 standard deviations (SD) from the mean were removed, and standardization was performed using the *scale* function in R, including centering and scaling. Subsequently, the PCA analysis was performed using SPSS software, and the corresponding sediment plot was generated (see Supplementary Fig. 1).

Then, the influence of major confounding factors on proteomic outcomes in both plasma and CSF was assessed by examining their correlation with the top six principal components (PC1-6), which collectively captured 30% of the overall experimental variance (see Supplementary Table 3). In this analysis, Spearman's rho coefficients were computed to measure the relationships between each of the six principal components and several potential confounders or key phenotypes of interest using SPSS. The results of this analysis were then presented in a heatmap figure created with the Heatmapper tool (Babicki et al., 2016)(see Supplementary Fig. 2*)*. This analysis allowed us to gain insights into the potential confounding factors affecting our experiments. Specifically, PC1 and PC4 exhibited statistically significant

correlations with age, while PC2 and PC3 demonstrated a strong correlation with the duration of sample preservation in the freezers (as depicted in Supplementary Fig. 2). As a consequence, we decided to retain PC1-6 as covariates for use in subsequent downstream analyses. Controlling our studies by PC1-6 allowed us to manage the main aspects related to sample preservation and the age- or sex-related differences in protein values unrelated to the disease, which might otherwise perturb the observation of protein level effects in key phenotypes. The Q-albumin (Q-alb) index, also known as the albumin quotient, is a measure used in medical diagnostics to assess the integrity of the blood-brain barrier (BBB). It is calculated by comparing the concentration of albumin in the cerebrospinal fluid (CSF) to the concentration of albumin in the blood plasma. We deconvoluted Q-alb into its two components and analyzed them in the context of the principal component analysis (PCA). In fact, albumin in blood was associated with PC4 (Supplementary Fig. 2). Our main findings are controlled by PC1-6, and therefore, potential confounding of Q-alb is well controlled by PC1-6 covariates.

c. Hierarchical clustering for the identification of highly correlated plasma-CSF protein levels

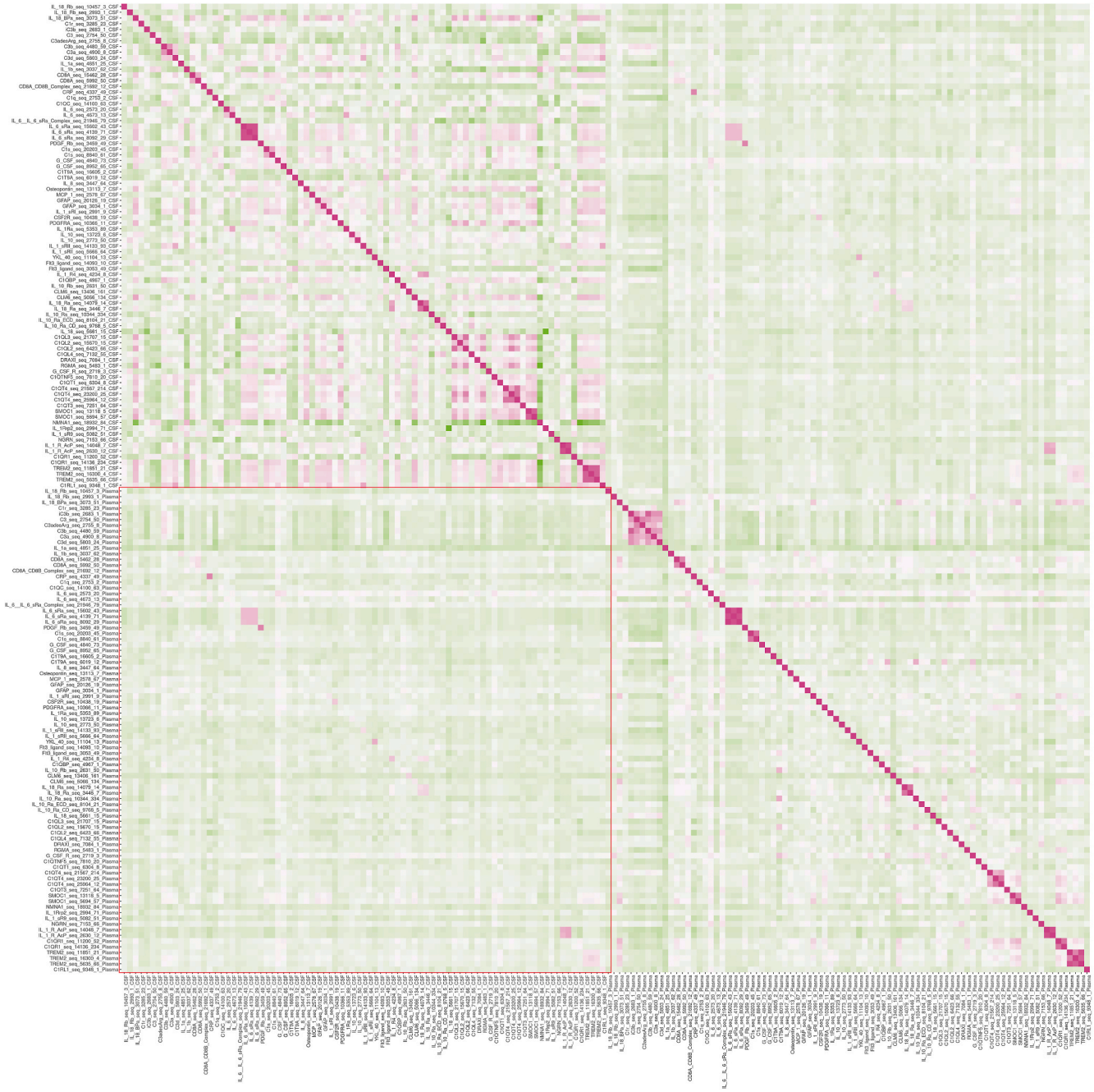

Fig. 1. Global overview of pairwise comparisons involving Euclidean proximities calculated during the NNC analysis. We conducted this analysis on a subset of 485 individuals, carefully selected for having all protein measurements registered in both plasma and CSF, thus eliminating the need for any data imputation. NNC dendrogram generated by SPSS permitted the identification of the paired plasma-CSF protein levels with proximity scores below 400. The blue square highlights the plasma-CSF comparisons that appear considerably more distant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

To identify the protein pairs with high concordance between plasma and CSF, we employed an unsupervised strategy based on a well-established clustering methodology. Specifically, we conducted clustering using the squared Euclidean distance, a common technique in data analysis, particularly in unsupervised machine learning and data mining (Cui et al., 2022). This method groups data points into clusters based on their similarity or dissimilarity, using the squared Euclidean distance as a measure of dissimilarity between data points. In this process, we computed the scoring values (with 0 being the null distance between two variables) among the measurements of plasma and CSF proteins using the SPSS distance function *Euclidian proximities*. We conducted this analysis on a subset of 485 individuals, carefully selected for having all protein measurements registered in both plasma and CSF, thus eliminating the need for any data imputation. As shown in Supplementary Table 4, the results generated a global proximity matrix used for conducting a nearest-neighbor clustering (NNC) of proteins. This method is a relatively simple and intuitive approach to clustering data points based on their proximity to their nearest protein neighbors.

The inspection of the global proximity matrix and the NNC dendrogram generated by SPSS (see Supplementary Fig. 3) permitted the identification of the paired plasma-CSF protein levels with proximity scores below 400 (boxed in red in Supplementary Table 4). The selection of highly correlated plasma-CSF proteins was subsequently validated in the remaining subset of 873 individuals using conventional correlation analyses with Spearman's rho coefficients in SPSS. To assess the overall significance of the proteins with strong correlations between central and peripheral compartments, we further calculated Spearman coefficients across the entire cohort of 1369 subjects. The pairwise global proximity matrix figure was built using Heatmapper software (Babicki et al., 2016) (see Fig. 1).

d. Statistical analysis of MCI to dementia progression and evaluation of other AD endophenotypes

To assess the prognostic value of the selected plasma and CSF proteins, we applied an analysis scheme identical to that defined in our previous publication (Orellana et al., 2022) to a cohort of 721 MCI subjects who had SOMAscan proteomic data and available follow-up information. We evaluated the impact of these selected proteins (six protein levels in two compartments) on the risk of transitioning from MCI to dementia using Cox proportional hazard regressions. These regressions were adjusted for factors including age, sex, baseline Mini-Mental State Examination (MMSE) score, A^+ (indicating amyloid positivity in the brain), and T^+ (indicating p-tau positivity), utilizing the previously established cutoff values of CSF AD biomarkers in our laboratory. Additionally, the Cox models incorporated the six principal components of protein variance that were previously calculated. We set the corrected significance level at alpha = 0.05/12 = 0.004. Furthermore, we delved into the raw and adjusted relationships between the selected proteins and AD endophenotypes, such as the clinical disease stage age at onset of symptoms, sex, *APOE* genotype status, ATN status, and MMSE scores, employing conventional statistical analysis within the SPSS software. Finally, to assess the robustness of the results obtained in disease progression, a series of sensitivity analyses were conducted. These included stratification by amyloid status and comparison of the full model selected for this analysis with alternative models: one without covariates and others with the exclusion of a single covariate (MMSE, age, sex, or AD CSF biomarkers).

4. Access to publicly available databases

To investigate the connection between selected proteins and AD, we utilized the STRING database (Szklarczyk et al., 2023). The STRING database (https://string-db.org/) systematically compiles and integrates protein-protein interactions, including both physical interactions and functional associations. The data are sourced from various origins, including automated text mining of scientific literature, computational predictions based on co-expression and conserved genomic context, databases of interaction experiments, and curated pathways/complexes from established sources.

We also accessed the Neurogenomics and Bioinformatics Center at Washington University in St. Louis, Missouri, USA (https://neurogenomics.wustl.edu/open-science/resource-sharing-hub/) to seek independent cross-sectional replications of the main findings observed in this study.

3. Results

3.1. Identifying proteins in CSF and plasma associated with the disease continuum

We obtained the plasma and CSF proteome profiles of 1369 consecutive individuals from the ACE CSF cohort using the SOMAscan platform. The mean age at the time of LP was 72.7 ± 8.0 years. The majority of the individuals (n = 833, 62.2%) had a clinical diagnosis of MCI, and more than half of them (>53.5%) exhibited evidence of hallmarks of AD pathology in the molecular analysis of CSF. Table 1 depicts the basic demographics of this large cohort.

To identify plasma and CSF proteins associated with dementia in our cohort, we conducted a comparative analysis of the mean levels of the 85 selected somamers (see Supplementary Table 1) across the three clinical categories of the disease continuum: cognitively normal, MCI, and dementia groups. The results of the ANOVA tests are presented in Supplementary Tables 5 and 6

As expected, a subset of inflammatory biomarkers in CSF (18 out of 85, 18.82%), corresponding to 14 unique proteins, exhibited statistically significant differences between groups after Bonferroni correction ($p < 2.65E-04$) (see Table 2). Notably, the CSF GFAP somamers showed the strongest association with the stages of the cognitive continuum (seq.20126.19, p = 4.26E-14), followed by SPARC-related modular calcium-binding protein 1 (SMOC1) (seq.5694.57, p = 2.10E-09) and sTREM2 (seq.11851.21, p = 3.505E-08) (Bonomi et al., 2023; Benedet et al., 2021). Importantly, the majority (15 out of 18, 83.3%) of statistically significant CSF somamers exhibited higher levels in the dementia group than in the MCI and cognitively normal groups. These observed asymmetries align with previous findings in other proteomic profiles and are attributed to the substantial proteome signature associated with tau levels, commonly observed in individuals with neurodegenerative conditions (Bader et al., 2020).

In plasma, 9 somamers were identified corresponding to 7 unique proteins that displayed statistically significant differences among the different clinical stages. Notably, the plasma-detected somamer showing the most prominent association with the disease continuum was SMOC1 (seq. 5694.57), with plasma levels approximately four orders of magnitude greater than CSF levels. Furthermore, it is worth noting that 5 plasma somamers, corresponding to 4 unique proteins (SMOC1, osteopontin, IL1R-RI, and C1QT4), exhibited consistency in terms of statistical significance, effect size, and directional trends when compared with somamers previously identified as significant in the CSF compartment. This suggests the presence of an overlapping proteomic signature related to the cognitive continuum between plasma and CSF, as detailed in Table 2.

However, it is important to note that none of the selected analytes displayed a strong correlation between plasma and CSF levels (rho <0.29; see Table 2). This lack of correlation may suggest different inflammatory processes occurring in both compartments. Alternatively, this behavior could be influenced by technical factors or as-yet-undiscovered biological processes governing both compartments.

We also examined the potential utility of these proteins in monitoring disease progression to dementia in MCI subjects (Table 2). We employed Cox proportional hazard risk models, adjusted for age, sex, baseline MMSE status, AT(N) status, and principal components, to

Table 1
Demographics of the ACE CSF cohort investigated in this study.

	Units	Missingness/effective sample	all	Dementia	MCI	Controls
N	Number	29/1369	1340	393	833	114
Age (mean, SD)	years	30/1339	72.7 (8)	74.8 (7.5)	72.8 (8)	66.7 (6.7)
Sex	% females	29/1340	57.8	64.4	54.5	59.6
APOE ε4 alleles (0,1,2)	Number (dosage)	61/1308	853, 393,62	86,23,3	531,240,41	236,130,18
Baseline MMSE score (mean, SD)	Score (0–30)	78/1291	24.5 (4.4)	20.6 (4.5)	25.7 (3.2)	29.4 (1)
Education (mean, SD)	Years of education	68/1301	8.3(4.8)	6.8(4.0)	8.3(4.7)	13.7(4.2)
BMI (mean,SD)	kg/m2	92/1277	26.9(4.3)	26.9(4.7)	27(4.2)	26.2(3.8)
CSF Aβ42 levels (mean, SD)	pg/ml	30/1339	818(399)	690(319)	844(415)	1070(376)
CSF p-tau levels (mean, SD)	pg/ml	30/1339	72(45)	85.5(54.5)	69.2(40.2)	45.2(18.9)
A+ (% amyloidosis)	% positive	30/1339	53.5	68.6	50.8	21.1
T+ (% p-tau positivity)	% positive	30/1339	53.8	64.3	53.7	18.4

Table 2
SOMAscan analytes that are statistically significant in cross-sectional comparisons across different strata of the cognitive continuum in plasma, CSF, or both compartments, and their association with disease progression (MCI to dementia conversion).

Somamers	CSF (ANOVA p-value)	Plasma (ANOVA p-value)	Spearman rho	Cox results CSF(HR, p)	Cox results plasma (HR, p)
GFAP.seq.20126.19	4.260E-14	4.504E-01	na	na	na
GFAP.seq.3034.1	2.454E-11	8.747E-01	na	na	na
SMOC1.seq.5694.57	2.100E-09	1.488E-12	0.095	1.306, p = 0.001	1.371, p = 0.003
TREM2.seq.11851.21	3.505E-08	5.363E-03	na	na	na
C1QT1.seq.6304.8	1.864E-07	5.873E-04	na	na	na
TREM2.seq.16300.4	4.248E-07	1.260E-03	na	na	na
IL.1Rrp2.seq.2994.71	7.599E-07	5.526E-01	na	na	na
IL.8.seq.3447.64	5.851E-06	3.249E-03	na	na	na
SMOC1.seq.13118.5	9.132E-06	2.779E-10	0.102	1.144, p = 0.07	1.283, p = 0.003
TREM2.seq.5635.66	9.996E-06	2.319E-03	na	na	na
Flt3.ligand.seq.14093.10	1.555E-05	3.237E-01	na	na	na
NMNA1.seq.18932.84	1.743E-05	6.565E-01	na	na	na
Osteopontin.seq.13113.7	4.868E-05	2.000E-05	0.101	0.976, p = 0.721	1.073, p = 0.229
IL.1.sRI.seq.2991.9	5.004E-05	2.166E-04	0.281	1.033, p = 0.665	1.061, p = 0.472
C3a.seq.4900.8	5.047E-05	1.644E-03	na	na	na
C1s.seq.8840.61	5.522E-05	6.324E-01	na	na	na
C1s.seq.20203.45	1.441E-04	2.029E-01	na	na	na
C1QT4.seq.23200.25	2.533E-04	2.356E-04	0.082	0.849, 0 = 0.029	0.996, p = 0.963

Note: CSF and plasma protein levels associated with disease status (ANOVA), plasma-CSF intercompartmental correlations (Spearman rho), and disease progression (Cox results). Red numbers indicate study-wide statistically significant findings.

control for experimental biases. In this analysis, most of the somamers, whether in plasma or CSF, did not display Bonferroni-corrected significant associations with disease progression (Bonferroni correction p = 0.005 for 20 comparisons).

However, it is worth noting that SMOC1 levels in both plasma and CSF showed study-wide associations with disease progression (0.003 > p > 0.001) (somamer SMOC1.seq.5694.57, Table 2). Although both CSF and plasma SMOC1 levels were correlated with CSF amyloid and p-tau levels—particularly CSF SMOC1 levels with p-tau (p < 2.2E-16) (see Supplementary Figs. 4 and 5)—the associations with disease progression persisted even after adjusting for protein levels in the central compartment, p-tau, amyloid positivity, and modeling SMOC1 levels in both compartments (CSF and plasma) simultaneously within the same statistical model. This suggests that the effects of SMOC1 levels on disease progression observed in the two compartments may reflect different molecular mechanisms independent of the primary hallmarks of AD pathology.

3.1.1. Exploring highly correlated proteins between plasma and CSF compartments using nearest-neighbor clustering

Next, to identify the most highly correlated proteins between the plasma and CSF compartments, we conducted an unsupervised nearest-neighbor clustering (NNC) analysis, as illustrated in Supplementary Fig. 3, using proteome profiles from 485 individuals with complete results for the selected proteins. Notably, the CSF-CSF comparisons reveal a high degree of correlation among multiple independent CSF measurements (Fig. 1). This finding strongly supports the existence of a distinct signature affecting multiple proteins, occurring exclusively within the CSF compartment.

In contrast, the scores for the plasma-plasma and plasma-CSF comparisons appear considerably more distant. This observation suggests that the relationships between protein measurements in the plasma and those in the CSF are less correlated compared with the cohesive patterns seen within CSF measurements. These results offer valuable insights into the distinctive nature of CSF protein interactions compared with those in the plasma (Fig. 1).

Through this analysis, we identified 10 somamers corresponding to 6 different proteins (CRP, sIL6RA, sPDGFRB, YKL-40, sIL1RL1, and sIL1-RAP) that displayed strong proximity by clustering both the CSF and blood compartments together (CSF-plasma pairs with proximity scores below 400 are detailed in Supplementary Table 4). To reinforce our findings regarding the central and peripheral compartmental correlations of these proteins, we calculated Spearman's rho correlations of identified CSF-plasma somamer pairs. As expected, the set of somamers selected using NNC exhibited robust Spearman correlation coefficients (n = 485, rho >0.518)(Table 3).

These correlation results were subsequently validated using the remaining subcohort (n = 884, rho >0.512). Spearman correlation analyses were extended to encompass the entire dataset, revealing that all observed correlations significantly surpassed the Bonferroni correction threshold (rho >0.519, p < 1.77E-93, as illustrated in Table 3). Scatter plots were also generated for the selected proteins (see Fig. 2). These results further underscore the highly significant correlation between the CSF and plasma levels of these specific proteins. Notably, we observed a clear bimodal distribution pattern in two proteins, YKL-40 and sPDGFRB, which may indicate the presence of genetic variants

Table 3
Spearman correlation coefficients of identified somamers with strong CSF-plasma proximity.

Somamer	Rho (NCC)	Rho (validation)	Rho (global)	p-value
Sample size (n)	**485**	**884**	**1369**	
CRP.seq.4337.49	0.872	0.841	0.852	<1E-303
IL.6.sRa. seq.15602.43	0.662	0.523	0.574	3.1311E-91
IL.6.sRa. seq.4139.71	0.66	0.513	0.576	2.677E-120
IL.6.sRa. seq.8092.29	0.653	0.535	0.577	6.346E-121
PDGF.Rb. seq.3459.49	0.788	0.806	0.8	7.85E-303
YKL.40. seq.11104.13	0.64	0.659	0.653	4.802E-166
IL.1.R4.seq.4234.8	0.52	0.518	0.519	1.7655E-93
IL.1.R.AcP. seq.14048.7	0.665	0.599	0.616	1.593E-141
IL.1.R.AcP. seq.2630.12	0.685	0.607	0.636	4.832E-154

Note: Rho (NCC) for the discovery dataset (n = 485). Rho (validation) indicates Spearman coefficient results in the confirmatory experiment with 884 additional samples. Rho (global) indicates the global results (NCC + validation, n = 1369). The p-value shown is for the global results.

influencing their expression. We also plotted the fitted lines for clinical category subgroups. Our results suggest that CSF-plasma correlations remain unaffected by the disease status within our series.

3.1.2. Soluble interleukin-6 receptor alpha subunit (sIL6RA) levels are associated with MCI conversion to dementia

After identifying and validating proteins with a strong plasma-CSF correlation, we proceeded to assess their potential involvement in the disease continuum through ANOVA analysis and their role in the progression from MCI to dementia using adjusted Cox proportional hazard models (see Table 4). For these analyses, we established a study-wide significance threshold of $p < 0.00277$, considering the 18 independent comparisons (somamers), involving 6 proteins within two distinct compartments. Interestingly, none of the NNC proteins showed a significant association with the disease continuum. The YKL-40 protein levels in plasma emerged as the most noteworthy, displaying a nominal statistical association with a p-value of 0.004.

In contrast, when we applied the adjusted Cox proportional hazard risk models, the plasma sIL6RA protein levels showed a strong and statistically significant association with disease progression. For each standard deviation increase in sIL6RA levels, there was a substantial 93.6% rise in the risk of conversion, yielding a hazard ratio (HR) of 1.936 with a 95% confidence interval (CI) of [1.276–2.935] and a p-value of 0.00187 (Fig. 3C). It is noteworthy that, despite the high correlations detected between plasma and CSF levels of sIL6RA (rho = 0.574), the association with disease progression appears to be specific to the protein's circulating plasma levels ($p > 0.422$ for sIL6RA CSF levels). To check the specificity of these findings for Alzheimer's disease (AD), we stratified the results by amyloid status (Supplementary Table 7). Interestingly, plasma sIL6RA shows a more specific association with AD dementia, particularly in amyloid-positive individuals. These findings suggest that sIL6RA might be a more specific marker for AD. The observed results were further scrutinized with additional sensitivity analyses, which revealed that the associations of plasma sIL6RA with disease progression were robust across various models (Supplementary Table 8a).

Of note, the plasma levels of sIL6RA exhibited no statistically significant associations with other crucial factors linked to dementia, including age ($p = 0.626$), CSF amyloid levels ($p = 0.468$; Fig. 3a), CSF p-tau levels ($p = 0.376$; Fig. 3b), the *APOE* genotypes ($p = 0.189$), and MMSE score ($p = 0.725$, Supplementary Fig. 6). These results reaffirm that plasma sIL6RA protein is an independent factor modulating MCI conversion to dementia. However, our analysis revealed modest sex-based differences in plasma sIL6RA levels within our study population, with a difference of 0.17 standard deviations favoring higher mean sIL6RA values in females ($p = 0.00094$; Fig. 3D). This last observation has important implications for plasma sIL6RA modeling in future studies.

3.1.3. Connecting identified proteins with AD pathogenesis using protein-protein interaction analysis (ppi) and their relation with APOE genotype

To further explore the association between plasma and CSF levels of SMOC1, IL6 pathways, and AD, protein–protein interaction (PPI)

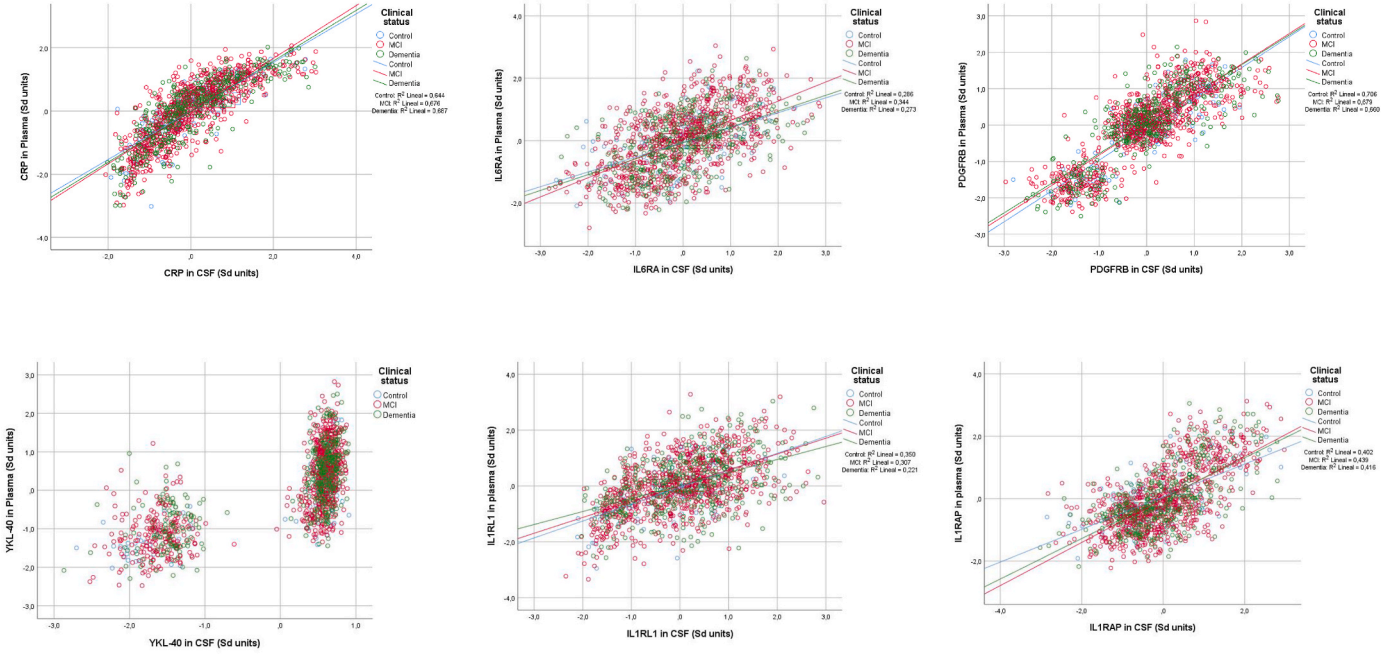

Fig. 2. Scatterplots of proteins demonstrating highly significant correlations between plasma and CSF compartments (n = 1369).

Table 4
Somascan analytes (somamers) showing high CSF-plasma concordance using NNC and its relation with disease status.

Somamer	CSF (ANOVA, p)	Plasma (ANOVA, p)	Spearman (rho)	Cox results CSF (HR, p)	Cox results plasma (HR, p)
CRP.seq.4337.49	0.654	0.853	0.852	1.04, p = 0.771	0.933, p = 0.583
IL.6.sRa.seq.15602.43	0.86	0.301	0.574	0.979, p = 0.817	**1.936, p = 0.00187**
IL.6.sRa.seq.4139.71	0.01	0.381	0.576	1.078, p = 0,422	1.428, p = 0.061
IL.6.sRa.seq.8092.29	0.31	0.464	0.577	1.003, p = 0.974	1.558, p = 0.042
PDGF.Rb.seq.3459.49	0.941	0.492	0.8	1.318, p = 0.039	0.805, p = 0.11
YKL.40.seq.11104.13	0.562	0.004	0.653	0.839, P = 0.045	1.204, p = 0.046
IL.1.R4.seq.4234.8	0.023	0.126	0.519	0.992, p = 0.926	1.069, p = 0.467
IL.1.R.AcP.seq.14048.7	0.431	0.951	0.616	0.926, p = 430	1.105, p = 0.315
IL.1.R.AcP.seq.2630.12	0.282	0.989	0.636	1.021, p = 0.757	0.988, p = 0.908

Note: Somamers showing high CSF-plasma concordance using NNC and its relation with disease status (ANOVA tests) or disease progression (Cox results). Red numbers indicate study-wide statistically significant findings.

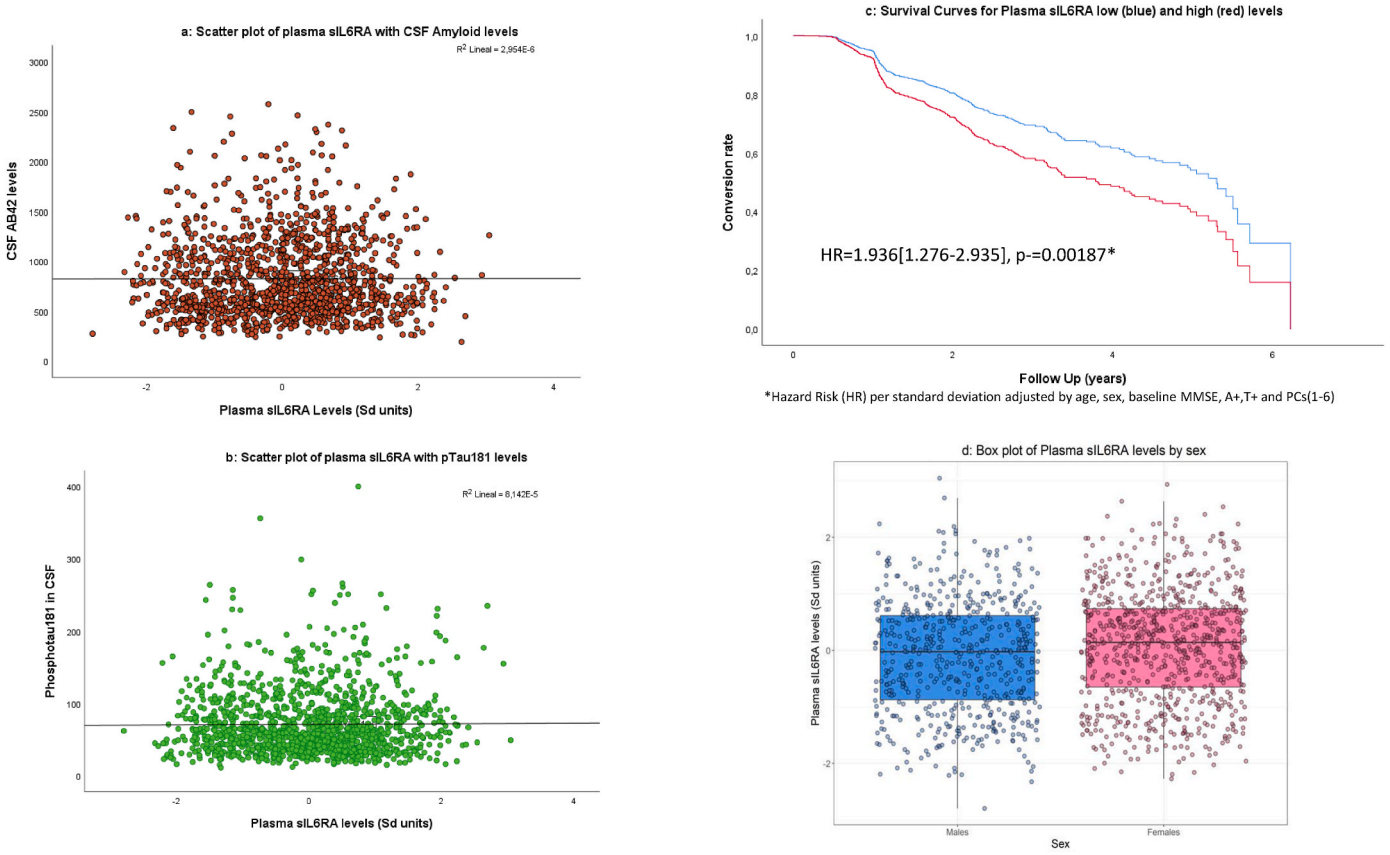

Fig. 3. Plasma sIL6RA levels and their relationships with amyloid, p-tau, MCI to AD conversion, and sex. Horizontal lines presented in panels a and b show the lack of significant associations of plasma sIL6RA levels with CSF amyloid levels or CSF p-tau levels.

networks were generated using STRING v11.064 (Szklarczyk et al., 2023). The STRING database integrates all known and predicted associations between proteins, including both physical interactions and functional associations. Pathway analysis of SMOC1 and IL6, along with six proteins showing high CSF-plasma concordance and their interactions with APP and MAPT, revealed a highly significant degree of protein–protein interactions (p = 3.37 × 10−6). However, SMOC1 did not show any PPIs with the selected proteins (Supplementary Fig. 7). Additionally, we explored the role of all proteins studied in either CSF or plasma in relation to the most relevant AD genetic risk factor (*APOE* gene). Partial correlation analyses revealed that none of the proteins were significantly associated with the *APOE* epsilon 4 genotype (p > 0.0005 after Bonferroni correction) (Supplementary Tables 9 and 10).

4. Discussion

In this study, we analyzed 63 inflammatory-related protein levels in paired plasma and CSF samples from 1369 individuals of a memory clinic to identify associations between plasma and CSF compartments and find inflammatory proteins associated with AD progression. We scrutinized 85 selected somamers related to inflammation, identifying 18 CSF somamers linked to disease status, notably including GFAP, SMOC1, and sTREM2. The majority of significant CSF somamers (83.3%) exhibited elevated levels in individuals with dementia. In plasma, we also found 9 somamers associated with the clinical continuum, with SMOC1 being the most prominent one.

The unsupervised NNC analysis revealed a striking phenomenon: high correlations between multiple proteins were exclusively observed within the CSF compartment (Fig. 1). This result aligns with prior findings showing that numerous inflammatory proteins in CSF displayed

similar patterns in AD patients and cognitively normal subjects (Boström et al., 2021). One plausible explanation for the substantial co-overexpression of these inflammatory proteins in CSF is the potential involvement of a generalized immunological response to the deposition of amyloid and tau proteins in the brain. Indeed, when we applied post hoc analyses to correct the ANOVA results by accounting for age and AD biomarkers, the previously observed associations with the disease continuum in CSF disappeared. The adjusted results revealed no significant associations of CSF proteins with the disease continuum ($p > 0.05$). This post hoc analysis underscores the intricate interconnections and dependencies among the multiple associations detected in the CSF proteome.

Notably, we observed a similar reduction in associations for most plasma proteins related to the clinical continuum after adjusting for AD CSF biomarkers (amyloid Aβ42 and p-tau levels). Nevertheless, the plasma proteins identified, including SMOC1, IL18, sIL18R, osteopontin, sIL1R1, CTRP4, and CD93 (C1QR), whether individually or in combination, have the potential to serve as blood-based surrogates for assessing AD pathology. It is worth noting that we remain cautious about their ability to surpass the performance of the plasma biomarkers currently under evaluation, such as several phosphorylated tau isoforms (p-tau181, p-tau217, p-tau231) and Aβ42/Aβ40 levels (Ashton et al., 2023; Blennow et al., 2023). Nonetheless, this working hypothesis merits further exploration in future investigations.

Another conclusion drawn from the NNC analysis is a general dissociation between the plasma and CSF compartments for most of the studied proteins (see boxed area in Fig. 1). This finding aligns with previous research, which has consistently shown a limited correlation between peripheral and central compartments in various studies (Whelan et al., 2019b; Bettcher et al., 2018). Furthermore, the relationships between these compartments may also be influenced by the permeability of the blood-brain barrier (BBB)(Dayon et al., 2019). Recent studies have indicated that changes in BBB function, potentially resulting in altered permeability and transport, are associated with the expression of specific inflammatory proteins (Takata et al., 2021). Moreover, the shifting distribution of these proteins from the bloodstream to the CSF in individuals with MCI and AD dementia might be linked to more pronounced disruptions in BBB permeability (Ott et al., 2018).

Building upon this concept, we investigated the impact of total protein and albumin levels in both CSF and plasma as general factors influencing protein measurements in these compartments. Interestingly, we found that only albumin levels in blood emerged as a significant factor impacting the levels of the selected proteins in this study. Specifically, plasma albumin levels were associated with PC4, affecting the variance of the proteins under study (Supplementary Fig. 2). We suggest that such adjustments should be considered in future studies as well.

Despite the observed limited concordance between the CSF and plasma compartments, the NNC analysis successfully identified six proteins (CRP, IL1RAP, ILRL1, sIL6RA, PDGFRB, and YKL-40) represented by 9 somamers with levels displaying a significant agreement between central and peripheral compartments. These results were confirmed and extended in subsequent analyses, as detailed in Table 3. While we cannot rule out any specific hypothesis, several plausible explanations can be considered based on the molecular characteristics and biological behavior of these proteins. Firstly, the molecular weight of these proteins and their ability to interact with endothelial receptors suggest that their correlation between the periphery and the CNS does not necessarily depend on their selective ability to cross the blood-brain barrier (BBB) or alterations in the BBB. This indicates that other mechanisms may be at play. One possible explanation, is that these mediators are produced at high levels by inflammatory cells in both compartments, such as microglia in the CNS and macrophages in the periphery. This could lead to a similar expression pattern in both plasma and CSF, resulting in the observed correlation. A more intriguing hypothesis is that these markers are produced by inflammatory endothelial cells present in the BBB and other vessels throughout the body. Supporting this view, Platelet-Derived Growth Factor Receptor Beta (PDGFRB) is highly expressed in vascular smooth muscle cells and pericytes under inflammatory conditions. This expression can lead to the release of PDGFRB as a soluble receptor in both CSF and plasma. Additionally, proteins such as YKL-40, C-Reactive Protein (CRP), Interleukin-6 Receptor Alpha (IL6RA), and Interleukin-1 Receptor Accessory Protein (IL1RAP) are known to be expressed at high levels in inflamed endothelial cells, vascular smooth muscle cells, or pericytes in various pathologies.

However, it is worth noting that, with a single exception (sIL6RA), the proteins showing high CSF-plasma concordance displayed little or no potential either as cross-sectional biomarkers for AD or as longitudinal biomarkers for disease progression, as summarized in Table 4. Thus, it is evident that a robust correspondence between plasma and CSF levels of these proteins does not necessarily ensure their utility as AD biomarkers in subsequent analyses. Overall, the results suggest that sIL6RA is more specific for AD dementia, with an increased effect in amyloid-positive (A+) individuals. In contrast, the association of plasma and CSF SMOC1 levels with disease progression is homogeneous across the cohort, irrespective of amyloid status, suggesting a more general role in dementia progression not strictly related to Alzheimer's disease diagnosis (see Supplementary Table 7).

One of the most intriguing findings of this study pertains to the levels of SMOC1 in both plasma and CSF. This protein, encoded by a developmental gene linked to the Waardenburg anophthalmia syndrome (Abouzeid et al., 2011), has been associated with TGF-beta signaling in the glomerulus, with its regulation involving nitric oxide (Dreieicher et al., 2009). While during embryonic development, SMOC1 expression levels are generally high across multiple tissues involved in organogenesis and development, in adults, studies indicate that SMOC1 may be more abundantly expressed in certain regions of the brain, such as the cerebral cortex, cerebellum, and hippocampus. This asymmetric distribution together with a high molecular weight (100 kDa) could explain the lack of a statistically significant correlation between SMOC1 levels in CSF and plasma.

In the context of AD and related dementias, previous small-scale proteome analyses have shown associations of this protein in both central and peripheral compartments with disease status. For instance, SMOC1 exhibited associations with disease status when measured in serum (Dammer et al., 2022), CSF (Zhou et al., 2020; Watson et al., 2023), human brain tissues (Bai et al., 2020; Sathe et al., 2021), and the amyloid plaque proteome (Drummond et al., 2022; Zaman et al., 2023). Most notably, SMOC1 was found to be elevated in CSF nearly 30 years before the onset of symptoms in individuals carrying Mendelian (autosomal dominant) mutations responsible for early-onset AD (Johnson et al., 2023). Therefore, our large-scale results validate previous cross-sectional observations, as we employ a very large independent cohort and a different proteomic platform. However, these cross-sectional associations vanished after accounting for covariation with biomarkers of AD pathology. This is unsurprising, given previous findings indicating that SMOC1 co-localizes with amyloid and tau deposits (Drummond et al., 2022; Zaman et al., 2023). Consequently, SMOC1 could be regarded as a potential early surrogate biomarker for AD pathology.

Of particular significance in our study is the novel finding regarding the association between SMOC1 levels in both CSF and plasma and the progression from MCI to dementia. Our analysis revealed that elevated SMOC1 levels in both compartments are significantly correlated with an increased risk of disease progression, as outlined in Table 3. Notably, these associations remained robust in sensitivity analyses (Supplementary Table 8) despite the relatively modest increase in risk (30% per standard deviation for CSF SMOC1 protein levels and 37% per standard deviation for plasma SMOC1 protein levels). These findings persisted even after adjusting for covariation with ATN biomarkers, age, sex, baseline MMSE, and principal components, and accounting for

SMOC1 protein levels in both CSF and plasma simultaneously.

We propose that CSF and plasma SMOC1 levels are independent risk factors for the progression of MCI to dementia. The lack of correlation between central and peripheral SMOC1 levels, along with their independence from each other, suggests that these measurements may be tracking distinct molecular processes. Alternatively, the presence of SMOC1 in both compartments could indicate the simultaneous tracking of BBB damage and amyloid deposition. We acknowledge the need for an independent confirmatory study to validate this observation. Interestingly, analyzing SMOC1 protein levels between AD cases and controls across multiple biofluids using an independent cohort from the Neurogenomics and Bioinformatics Center at Washington University in St. Louis (NGI database; https://neurogenomics.wustl.edu/open-science/resource-sharing-hub/) also supported the cross-sectional association of SMOC1 in brain, CSF, and plasma, further reinforcing the results observed in our study (Supplementary Table 11).

Another noteworthy finding in this study pertains to the levels of plasma sIL6RA protein, a receptor for the cytokine IL-6. IL-6 is a pivotal immunomodulatory cytokine implicated in the pathogenesis of autoimmune and chronic inflammatory diseases, among others. Importantly, IL-6 may exert its biological activities through two distinct modes of action. The classical IL-6 signaling involves its sequential binding to two receptors: a type I transmembrane glycoprotein termed IL6RA (CD126 or gp80) and the type I transmembrane signal transducer protein gp130 (CD130). Notably, IL6RA may also be partially proteolyzed by disintegrin and the metalloproteinases ADAM10 and ADAM17, yielding the solute form sIL6R, which binds IL-6 with the same affinity as membrane-bound IL6RA (Chalaris et al., 2010). The complex sIL6RA/IL-6 can also interact with gp130 and transduce intracellular signals through a process known as trans-signaling (Rose-John et al., 2023). Interestingly, the differential signal transduction route has a strong impact on the biological outcome of IL-6 signals. Whereas the classic signaling via membrane-bound IL6RA has homeostatic and protective effects, the sIL6RA-mediated trans-signaling is strongly associated with pathological inflammatory responses (Campbell et al., 2014).

The role of sIL6RA in AD and related dementias has been the subject of previous investigations. Initial studies reported discordant and statistically significant changes in sIL6RA levels in AD dementia patients compared with controls (Hampel et al., 1998; Bongioanni et al., 1998; Bagli et al., 2003). A potential impact of IL6 signaling on the transcription and expression of amyloid precursor protein has been suggested (Ringheim et al., 1998). More recent research found varying *IL6RA* expression levels in different regions of the brain (Hampel et al., 2005). One study detected elevated sIL6RA levels in plasma and CSF, which correlated with AD biomarkers and lower cognitive scores (Quillen et al., 2023).

Many of these earlier findings were based on small datasets and cross-sectional methodologies. However, our study, based on a larger dataset, did not support these cross-sectional effects of sIL6RA or its association with biomarkers of AD pathology (Fig. 3). In contrast, Cox proportional hazard models revealed a strong link between sIL6RA plasma levels and MCI-to-dementia conversion, indicating a 93.6% increased risk of conversion per standard deviation. This association was independent of the clinical syndromic continuum or AD biomarkers and demonstrated sexual dimorphism in plasma levels (Fig. 3). Importantly, this finding remained consistent even after adjusting for the full set of previously described covariates.

Mechanistically, it could be postulated that higher sIL6RA levels would favor pathological IL-6 trans-signaling. However, it is intriguing that sIL6RA levels are associated with disease progression only when measured in the peripheral compartment. Several explanations are possible. First, sIL6RA may be tracking an exclusive peripheral inflammatory component unrelated to AD pathology. Therefore, sIL6RA could be related to vascular (Montgomery et al., 2021; Choi et al., 2023) or metabolic (Theurich et al., 2017) comorbidities, accelerating the disease processes synergistically, as previously proposed (Power et al., 2018). An alternative explanation is that the central effects of sIL6RA in CSF might be overshadowed by the pervasive tau signature affecting numerous inflammatory mediators, as we have detected (Fig. 1).

While our study provides a comprehensive analysis of blood and CSF from 1369 patients using a multiplex proteomics-based SOMAscan screening assay with a selected panel of 63 candidate proteins, it is important to acknowledge a significant limitation. Although the paired analyses between blood and CSF offer valuable insights into correlative changes between peripheral and CSF compartments, the study's strength is limited by the lack of access to other databases that integrate paired CSF-plasma multiomics data. Given that the role of plasma sIL6RA levels in MCI progression represents a novel finding, independent validation of these results is necessary. To partially address this limitation, as we did with SMOC1, we analyzed differences in these protein levels between AD cases and controls across multiple biofluids in the NGI database. Notably, we observed significant differences only in plasma sIL6RA ($p = 0.019$) and CSF sIL1RAP ($p = 0.048$) (see Supplementary Table 11). The findings related to sIL6RA somewhat support our promising results. However, further confirmation by other research groups and validation using alternative protein detection technologies are crucial initial steps in establishing these new and promising biomarkers.

If confirmed, plasma sIL6RA levels could become a valuable tool for monitoring MCI progression and may open new avenues for AD therapeutics. Research on IL-6 in neuroinflammation and AD has led to the development of anti-IL-6 and IL-6R monoclonal antibodies (mAbs), such as tocilizumab, sarilumab, and satralizumab, designed to reduce chronic inflammation but not specifically targeting IL-6 trans-signaling, potentially affecting IL-6's beneficial roles. It is worth mentioning that selective IL-6 trans-signaling inhibitors are currently under clinical development, and olamkicept, a soluble gp130Fc variant, has shown promising results in phase II clinical studies for inflammatory bowel disease. Therefore, our findings on the IL-6 pathway might have significant clinical implications for AD, provided that causality and the underlying mechanisms are elucidated.

CRediT authorship contribution statement

Xavier Morató: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Raquel Puerta1:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation. **Amanda Cano:** Writing – review & editing, Writing – original draft, Validation, Data curation, Writing – review & editing, Writing – original draft, Visualization, Data curation. **Adelina Orellana:** Writing – review & editing, Data curation. **Itziar de Rojas:** Writing – review & editing, Writing – original draft, Validation, Data curation. **María Capdevila:** Writing – review & editing. **Laura Montrreal:** Writing – review & editing, Resources. **Maitée Rosende-Roca:** Writing – review & editing, Data curation. **Pablo García-González:** Writing – review & editing, Data curation. **Claudia Olivé:** Writing – review & editing, Data curation. **Fernando García-Gutiérrez:** Writing – review & editing, Data curation. **Josep Blázquez:** Writing – review & editing. **Andrea Miguel:** Writing – review & editing. **Raúl Núñez-Llaves:** Resources. **Vanesa Pytel:** Resources. **Montserrat Alegret:** Writing – review & editing, Data curation. **María Victoria Fernández:** Writing – review & editing. **Marta Marquié:** Writing – review & editing, Data curation. **Sergi Valero:** Writing – review & editing, Data curation. **Jose Enrique Cavazos:** Writing – review & editing, Supervision. **Santos Mañes:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Mercè Boada:** Writing – review & editing, Funding acquisition, Conceptualization. **Agustín Ruiz:** Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT-4 to enhance the readability of the text. After utilizing this tool/service, the author(s) reviewed the content, and professional services were used for proofreading as needed. The author(s) take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2024.100899.

Data availability

Data will be made available on request.

References

Abouzeid, H., et al., 2011. Mutations in the SPARC-related modular calcium-binding protein 1 gene, SMOC1, cause waardenburg anophthalmia syndrome. Am. J. Hum. Genet. 88, 92–98.
Ashton, N.J., et al., 2023. Plasma and CSF biomarkers in a memory clinic: head-to-head comparison of phosphorylated tau immunoassays. Alzheimers Dement 19, 1913–1924.
Babicki, S., et al., 2016. Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res. 44, W147–W153.
Bader, J.M., et al., 2020. Proteome profiling in cerebrospinal fluid reveals novel biomarkers of Alzheimer's disease. Mol. Syst. Biol. 16.
Bagli, M., et al., 2003. Polymorphisms of the gene encoding the inflammatory cytokine interleukin-6 determine the magnitude of the increase in soluble interleukin-6 receptor levels in Alzheimer's disease. Results of a pilot study. Eur Arch Psychiatry Clin Neurosci 253, 44–48.
Bai, B., et al., 2020. Deep Multilayer brain proteomics identifies molecular networks in Alzheimer's disease progression. Neuron 105, 975–991.e7.
Bellenguez, C., et al., 2022a. New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat. Genet. 54, 412–436.
Bellenguez, C., et al., 2022b. New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat. Genet. 54, 412–436.
Benedet, A.L., et al., 2021. Differences between plasma and cerebrospinal fluid glial fibrillary Acidic protein levels across the Alzheimer disease continuum. JAMA Neurol. 78, 1471–1483.
Bettcher, B.M., et al., 2018. Cerebrospinal fluid and plasma levels of inflammation differentially relate to CNS markers of Alzheimer's disease pathology and neuronal damage. J Alzheimers Dis 62, 385–397.
Blennow, K., et al., 2023. The potential clinical value of plasma biomarkers in Alzheimer's disease. Alzheimers Dement 19.
Bongioanni, P., Boccardi, B., Borgna, M., Ross, B., 1998. T-lymphocyte interleukin 6 receptor binding in patients with dementia of Alzheimer type. Arch. Neurol. 55, 1305–1308.
Bonomi, C.G., et al., 2023. Cerebrospinal fluid sTREM-2, GFAP, and β-S100 in Symptomatic Sporadic Alzheimer's disease: microglial, Astrocytic, and APOE Contributions along the Alzheimer's disease continuum. J Alzheimers Dis 92, 1385–1397.
Boström, G., et al., 2021. Different inflammatory signatures in Alzheimer's disease and frontotemporal dementia cerebrospinal fluid. J Alzheimers Dis 81, 629–640.
Campbell, I.L., et al., 2014. Trans-signaling is a dominant mechanism for the pathogenic actions of interleukin-6 in the brain. J. Neurosci. 34, 2503–2513.
Candia, J., et al., 2017. Assessment of Variability in the SOMAscan assay. Sci. Rep. 7.
Chalaris, A., et al., 2010. ADAM17-mediated shedding of the IL6R induces cleavage of the membrane stub by gamma-secretase. Biochim. Biophys. Acta 1803, 234–245.
Choi, B.R., Johnson, K.R., Maric, D., McGavern, D.B., 2023. Monocyte-derived IL-6 programs microglia to rebuild damaged brain vasculature. Nat. Immunol. 24, 1110–1123.
Cui, M., Cheng, C., Zhang, L., 2022. High-throughput proteomics: a methodological mini-review. Laboratory Investigation 2022 102, 1170–1181, 11 102.
Dammer, E.B., et al., 2022. Multi-platform proteomic analysis of Alzheimer's disease cerebrospinal fluid and plasma reveals network biomarkers associated with proteostasis and the matrisome. Alzheimer's Res. Ther. 14.
Dayon, L., et al., 2019. Proteomes of paired human cerebrospinal fluid and plasma: relation to blood-brain barrier permeability in Older adults. J. Proteome Res. 18, 1162–1174.
de Rojas, I., et al., 2021. Common variants in Alzheimer's disease and risk stratification by polygenic risk scores. Nat. Commun. 12.
Dreieicher, E., et al., 2009. Nitric oxide inhibits glomerular TGF-beta signaling via SMOC-1. J. Am. Soc. Nephrol. 20, 1963–1974.
Drummond, E., et al., 2022. The amyloid plaque proteome in early onset Alzheimer's disease and Down syndrome. Acta Neuropathol Commun 10.
Espinosa, A., et al., 2013. A longitudinal follow-up of 550 mild cognitive impairment patients: evidence for large conversion to dementia rates and detection of major risk factors involved. J Alzheimers Dis 34, 769–780.
Esteban De Antonio, E., et al., 2021. BIOFACE: a Prospective study of risk factors, cognition, and biomarkers in a cohort of individuals with early-onset mild cognitive impairment. Study Rationale and research protocols. J Alzheimers Dis 83, 1233–1249.
Gigase, F.A.J., et al., 2023. The association between inflammatory markers in blood and cerebrospinal fluid: a systematic review and meta-analysis. Molecular Psychiatry 2023 28, 1502–1515, 4 28.
Gold, L., et al., 2010. Aptamer-based multiplexed proteomic technology for biomarker discovery. PLoS One 5.
Hampel, H., et al., 1998. Decreased soluble interleukin-6 receptor in cerebrospinal fluid of patients with Alzheimer's disease. Brain Res. 780, 356–359.
Hampel, H., et al., 2005. Pattern of interleukin-6 receptor complex immunoreactivity between cortical regions of rapid autopsy normal and Alzheimer's disease brain. Eur Arch Psychiatry Clin Neurosci 255, 269–278.
Hansson, O., et al., 2021. The Alzheimer's Association international guidelines for handling of cerebrospinal fluid for routine clinical measurements of amyloid β and tau. Alzheimers Dement 17, 1575–1582.
Helmut, K., Hanisch, U.K., Noda, M., Verkhratsky, A., 2011. Physiology of microglia. Physiol. Rev. 91, 461–553.
Jack, C.R., et al., 2024. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup. Alzheimers Dement. https://doi.org/10.1002/ALZ.13859.
Johnson, E.C.B., et al., 2023. Cerebrospinal fluid proteomics define the natural history of autosomal dominant Alzheimer's disease. Nat Med 29, 1979–1988.
Jones, L., et al., 2015. Convergent genetic and expression data implicate immunity in Alzheimer's disease. Alzheimers Dement 11, 658–671.
Jonsson, T., et al., 2013. Variant of TREM2 associated with the risk of Alzheimer's disease. N. Engl. J. Med. 368, 107–116.
Juul Rasmussen, I., Frikke-Schmidt, R., 2023. Modifiable cardiovascular risk factors and genetics for targeted prevention of dementia. Eur. Heart J. 44, 2526–2543.

Kunkle, B.W., et al., 2019. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. Nat. Genet. 51, 414–430.
Le Guen, Y., et al., 2023. Multiancestry analysis of the HLA locus in Alzheimer's and Parkinson's diseases uncovers a shared adaptive immune response mediated by HLA-DRB1*04 subtypes. Proc Natl Acad Sci U S A 120.
Marquié, M., et al., 2023. The synergic effect of AT(N) profiles and Depression on the risk of conversion to dementia in patients with mild cognitive impairment. Int. J. Mol. Sci. 24.
McGeer, P.L., McGeer, E.G., 2013. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. Acta Neuropathol. 126, 479–497.
Montgomery, A., et al., 2021. Overlapping and distinct biological effects of IL-6 classic and trans-signaling in vascular endothelial cells. Am J Physiol Cell Physiol 320, C554–C565.
Orellana, A., et al., 2022. Establishing in-House cutoffs of CSF Alzheimer's disease biomarkers for the AT(N) stratification of the Alzheimer center Barcelona cohort. Int. J. Mol. Sci. 23.
Ott, B.R., et al., 2018. Blood-cerebrospinal fluid barrier Gradients in mild cognitive impairment and Alzheimer's disease: Relationship to inflammatory cytokines and chemokines. Front. Aging Neurosci. 10.
Power, M.C., et al., 2018. Combined neuropathological pathways account for age-related risk of dementia. Ann. Neurol. 84, 10–22.
Puerta, R., et al., 2023. Head-to-head comparison of aptamer- and antibody-based proteomic platforms in CSF samples of a real-world large cohort of a memory clinic. Alzheimer's Dementia 19.
Quillen, D., et al., 2023. Levels of soluble interleukin 6 receptor and Asp358Ala are associated with cognitive performance and Alzheimer disease biomarkers. Neurology(R) neuroimmunology & neuroinflammation 10.
Ringheim, G.E., et al., 1998. Enhancement of beta-amyloid precursor protein transcription and expression by the soluble interleukin-6 receptor/interleukin-6 complex. Mol. Brain Res. 55, 35–44.
Rodriguez-Gomez, O., et al., 2017. FACEHBI: a Prospective study of risk factors, biomarkers and cognition in a cohort of individuals with subjective cognitive decline. Study Rationale and research protocols. J Prev Alzheimers Dis 4, 100–108.
Rose-John, S., Jenkins, B.J., Garbers, C., Moll, J.M., Scheller, J., 2023. Targeting IL-6 trans-signalling: past, present and future prospects. Nat. Rev. Immunol. 23, 666–681.
Sathe, G., et al., 2021. Quantitative proteomic analysis of the frontal cortex in Alzheimer's disease. J. Neurochem. 156, 988–1002.
Sims, R., et al., 2017. Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. Nat. Genet. 49, 1373–1384.
Sun, Y.X., et al., 2003. Inflammatory markers in matched plasma and cerebrospinal fluid from patients with Alzheimer's disease. Dement. Geriatr. Cogn. Disord 16, 136–144.
Szklarczyk, D., et al., 2023. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 51, D638–D646.
Takata, F., Nakagawa, S., Matsumoto, J., Dohgu, S., 2021. Blood-brain barrier Dysfunction Amplifies the development of neuroinflammation: understanding of Cellular Events in brain Microvascular endothelial cells for prevention and Treatment of BBB Dysfunction. Front. Cell. Neurosci. 15.
Theurich, S., et al., 2017. IL-6/Stat3-Dependent Induction of a distinct, Obesity-associated NK cell Subpopulation Deteriorates Energy and Glucose Homeostasis. Cell Metab 26, 171–184.e6.
Tomé, S.O., Thal, D.R., 2021. Co-pathologies in Alzheimer's disease: just multiple pathologies or partners in crime? Brain 144, 706–708.
Tripathi, P., et al., 2024. Review of Pharmacotherapeutic Targets in Alzheimer's disease and its Management using Traditional Medicinal Plants. Degener. Neurol. Neuromuscul. Dis. 14, 47–74.
Walker, K.A., et al., 2023. The role of peripheral inflammatory insults in Alzheimer's disease: a review and research roadmap. Molecular Neurodegeneration 2023 18, 1–19, 1 18.
Watson, C.M., et al., 2023. Quantitative mass spectrometry analysis of cerebrospinal fluid protein biomarkers in Alzheimer's disease. Scientific Data 2023 10, 1–14, 1 10.
Whelan, C.D., et al., 2019a. Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer's disease. Acta Neuropathol Commun 7.
Whelan, C.D., et al., 2019b. Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer's disease. Acta Neuropathol Commun 7.
Zaman, M., et al., 2023. Dissecting Detergent-Insoluble proteome in Alzheimer's disease by TMTc-corrected Quantitative mass spectrometry. Mol. Cell. Proteomics 22.
Zhou, M., et al., 2020. Targeted mass spectrometry to quantify brain-derived cerebrospinal fluid biomarkers in Alzheimer's disease. Clin Proteomics 17.