

SHORT REPORT

CSF proteomic signature predicts progression to Alzheimer's disease dementia

Eleonora M. Vromen¹  | Marta del Campo Milán^{2,3} | Philip Scheltens¹ |
Charlotte E. Teunissen² | Pieter Jelle Visser^{1,4,5} | Betty M. Tijms¹¹ Alzheimer Center Amsterdam, Amsterdam Neuroscience, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands² Neurochemistry Laboratory, Department of Clinical Chemistry, Amsterdam Neuroscience, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands³ Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, CEU Universities, Urbanización Montepríncipe, Universidad San Pablo-CEU, Alcorcón, Spain⁴ Department of Psychiatry, Maastricht University, Maastricht, the Netherlands⁵ Department of Neurobiology, Care Sciences and Society, Division of Neurogeriatrics, Karolinska Institutet, Stockholm, Sweden

Correspondence

Eleonora M. Vromen, De Boelelaan 1118, 1081HZ Amsterdam, North Holland, the Netherlands.

E-mail: e.m.vromen@amsterdamumc.nl

Abstract

Introduction: Individuals in the Alzheimer's disease (AD) continuum with mild cognitive impairment (prodromal AD) are at increased risk to develop dementia. Still, underlying pathophysiological processes remain unclear. We studied whether cerebrospinal fluid (CSF) proteome changes are related to time to clinical progression in prodromal AD.**Methods:** We measured 671 CSF proteins in 49 prodromal AD individuals (67±7 years old, 22 [45%] female) from the Amsterdam Dementia Cohort. Associations of protein levels with time to dementia onset were tested with Cox regression models, followed by biological pathway enrichment analysis.**Results:** Eighteen (36%) individuals developed dementia during follow-up. In total, 128 (98%) proteins were associated with a 1.4- to 17-fold increased risk of progression to dementia (all $P < .05$). These proteins showed enrichment for immune system processes, signal transduction, neuronal death, and neurodevelopmental biology.**Discussion:** CSF proteome changes related to rate of progression to dementia can be detected in prodromal AD, providing more insight into processes involved in early AD pathophysiology.

KEYWORDS

Alzheimer's disease, cerebrospinal fluid, mild cognitive impairment, prognosis, proteomics

1 | INTRODUCTION

Alzheimer's disease (AD) is characterized by pathological depositions of amyloid beta (A β) plaques and hyperphosphorylated tau (neurofibrillary tangles) in the brain. Biomarkers for these pathological hallmarks can be measured in cerebrospinal fluid (CSF).¹ Individuals with mild cognitive impairment (MCI) and an abnormal amyloid biomarker (AD continuum stage 3), hereafter called prodromal AD individuals, are at increased risk to develop dementia.^{2,3} Individuals with prodromal AD show heterogeneity in disease progression rates, but the underlying

pathophysiological processes remain unclear. Recent advances in CSF proteomics provide the opportunity to study multiple biological processes at the same time within an individual. The first CSF proteomics studies comparing AD dementia individuals to controls have shown up- or downregulation of proteins related to different biological processes, including immune system processes, metabolism, hemostasis, and synaptic functioning.^{4,5} Such alterations may already be present in the MCI stage.⁵⁻⁷ Furthermore, MCI individuals who later progressed to AD dementia show CSF proteomic alterations compared to MCI individuals who remain stable.^{7,8} However, previous studies often

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* published by Wiley Periodicals, LLC on behalf of Alzheimer's Association

based diagnoses on clinical criteria without biomarker evidence of AD, and did not include time to dementia onset in their analysis. As such, it remains unclear which biological processes are related to rates of clinical progression in prodromal AD. In this study, we investigated whether CSF proteome changes can be detected that are related to progression to dementia in individuals with prodromal AD and which underlying biological processes are involved.

2 | METHODS

2.1 | Cohort

Prodromal AD individuals were included from the Amsterdam Dementia Cohort (ADC)⁹ when they had CSF proteomics and longitudinal clinical follow-up available. All met the National Institute on Aging–Alzheimer's Association (NIA-AA) criteria for MCI¹⁰ and had an abnormal baseline amyloid status as measured in CSF, reflecting AD continuum stage 3.³ Progression to dementia was assessed according to the NIA-AA criteria for AD dementia.¹¹ For this study, we labeled individuals as progression to AD dementia (MCI-p) or no progression (MCI-n) at follow-up. The study was approved by the medical ethical board of the institution and all participants gave written informed consent.

2.2 | CSF biomarkers

At first visit, a lumbar puncture was performed between the L3/L4, L4/L5, or L5/S1 intervertebral space using a 25-gauge needle and CSF was collected in polypropylene tubes. A β 1-42 (A β 42) and phosphorylated tau (p-tau) concentrations were determined using enzyme-linked immunosorbent assays (INNOTEST β -AMYLOID[1-42] and PHOSPHO-TAU[181P]; Fujirebio). A β 42 abnormality was determined using a drift-corrected cutoff of < 813 pg/mL.¹²

2.3 | CSF proteomics

CSF protein concentrations were quantified with multiplex panels based on the Proximity Extension Assay (PEA) technology (Olink Proteomics Inc.) as described previously.¹³ Briefly, 11 validated antibody-based protein panels were used, containing reagents to measure 979 proteins in total. Each protein was targeted by binding a unique pair of oligonucleotide-labeled antibody probes. A polymerase chain reaction (PCR) target sequence was formed for probes in close vicinity through a proximity-dependent DNA polymerization event. Maximizing sensitivity and specificity, this was further quantified with the Fluidigm BioMark HD real-time PCR platform, after which protein levels were log₂ transformed. Details on assay characteristics and validation can be found on the producer's website.¹⁴ Proteins were excluded from the analyses when they failed quality control or when they were detected in less than 10 individuals in either the MCI-p or MCI-n group, resulting in 671 (69%) unique proteins included for further analysis. To aid inter-

pretation, protein concentrations were Z-transformed to the mean and standard deviation of the whole group.

2.4 | Statistical analysis

Statistical analyses were performed using R v3.6.1 "Action of the Toes". Baseline comparisons between MCI-p and MCI-n were made with Chi-square tests, unpaired t-tests, or Mann-Whitney *U* tests. Associations of protein levels with time to dementia onset were tested with Cox proportional hazards models, adjusting for age, sex, and p-tau levels, as these are known to be related to time to dementia onset.¹⁵ Panther Gene Ontology (GO) and ClueGO were used for biological pathway enrichment analysis of all significant proteins.

3 | RESULTS

3.1 | Cohort demographics

We included 49 individuals (67 \pm 7 years old, 22 (45%) female; Table 1). Eighteen (37%) individuals progressed to AD dementia during a median follow-up of 2.1 [interquartile range: 1.2–3] years. There were no significant group differences in age, sex, apolipoprotein E (APOE) ϵ 4 allele carriers, or CSF A β 42 and p-tau levels.

3.2 | Proteomics for prediction of progression to dementia

In total, levels of 128 (19%) proteins were associated with time to dementia onset, of which 25 were also differentially expressed in MCI-p compared to MCI-n at baseline (Figure 1). The majority of 125 (98%) proteins had a hazard ratio (HR) below 1, indicating that lower concentrations of these proteins were associated with increased risks to progress to AD dementia (1.4- to 17-fold higher risk; Figure 1A, all *P* < .05). Proteins with the strongest association were ALDH3A1, GHRL, CBLIF, NOTCH1, and CD79B. Three proteins (RASA1, IL6, CCL18) had an HR above 1, indicating that higher concentrations of those proteins were associated with increased risks of progression to AD dementia (1.6- to 2.2-fold higher risk; Figure 1B, all *P* < .05). A model including only p-tau, age, and sex was not significant (*P* = .903).

3.3 | Pathway enrichment predicting progression to dementia

Next, we performed pathway enrichment analyses on proteins for which lower concentrations predicted progression to dementia using Panther GO and ClueGO, and found enrichment for biological processes associated with the immune system, including leukocyte activation and cytokine signaling, as well as signal transduction and the mitogen-activated protein kinase (MAPK) cascade, neuronal death,

TABLE 1 Baseline demographics for all individuals (n = 49)

| Characteristic | All (n = 49) | MCI-n (n = 31) | MCI-p (n = 18) |
|----------------------------------|---------------|----------------|----------------|
| Female sex, n (%) | 22 (45%) | 16 (52%) | 6 (33%) |
| Age, mean ± SD | 67.3 ± 6.8 | 67.5 ± 6.4 | 67.1 ± 7.8 |
| APOE ε4 carrier, n (%) | 32 (65%) | 21 (68%) | 11 (61%) |
| Follow-up time (y), median (IQR) | 2.1 (1.2-3) | 2.1 (1.1-3.1) | 2.1 (1.9-3) |
| Aβ42, median (IQR) | 600 (540-678) | 650 (527-699) | 589 (542-642) |
| P-tau, median (IQR) | 73 (50-98) | 73 (49-97) | 76 (54-101) |
| Abnormal p-tau, n (%) | 35 (71%) | 22 (71%) | 13 (72%) |

Note: No differences were observed between individuals based on progression to dementia status.

Abbreviations: Aβ42, amyloid beta 1-42; APOE, apolipoprotein E; IQR, interquartile range; MCI, mild cognitive impairment; MCI-n, MCI individuals without progression to AD dementia; MCI-p, individuals with progression to AD dementia; n, number; p-tau, phosphorylated tau; SD, standard deviation; y, years.

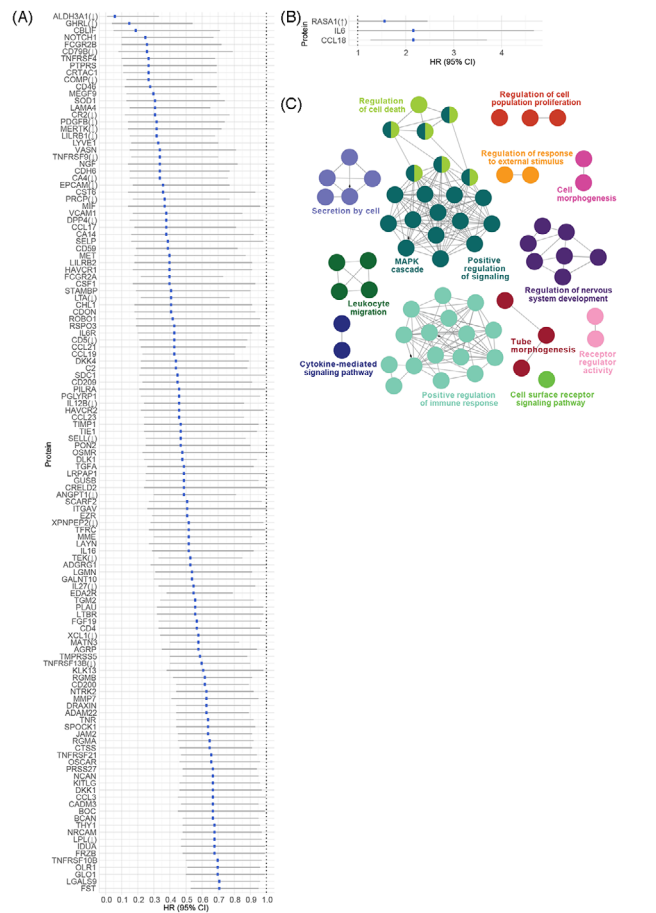


FIGURE 1 Association between proteins and time to dementia onset in prodromal AD individuals. A, Forest plot of proteins with a significant HR < 1 from Cox regression analysis adjusted for age, sex, and p-tau level. B, Forest plot of proteins with a significant HR > 1 from Cox regression analysis adjusted for age, sex, and p-tau level. (A+B) proteins with differential expression at baseline are marked with (↓) or (↑) for MCI-p compared to MCI-n (P-values < .05). C, Results from pathway enrichment analysis of all proteins with a significant HR < 1 using ClueGO for cluster visualization. AD, Alzheimer's disease; HR, hazard ratio; MCI, mild cognitive impairment; MCI-n, MCI individuals with no progression to AD dementia; MCI-p, MCI individuals with progression to AD dementia; p-tau, phosphorylated tau

and neurodevelopmental biology (Figure 1C, all pFDR < .05). We found no enrichment for proteins of which higher concentrations predicted progression to dementia.

4 | DISCUSSION

In this study, we identified a proteomic signature in CSF that was associated with a 1.4- to 17-fold increased risk of progression to AD dementia in prodromal AD. These proteins were involved in immune system processes, signal transduction, neurodevelopmental biology, and neuronal death, suggesting that these processes play a role in developing dementia.

Prior studies investigating proteome differences between MCI-n and MCI-p individuals cross-sectionally each reported 5 to 13 proteins to be differentially expressed, with little overlap between studies, and also with our results.^{7,8,16} One protein, NCAN, identified in our study was previously reported,⁷ but in an opposite direction. That previous study also showed a non-linear relationship between NCAN protein concentrations and cognitive status, with MCI individuals having higher protein concentrations compared to AD dementia individuals and cognitively normal individuals. Possibly, our MCI-p group may already display an “AD-like” profile compared to MCI-n.

We further extend the literature by taking time to clinical progression into account, and as a result observed a large group of proteins to be associated with progression to dementia. These showed enrichment for biological pathways involved in immune system processes and signal transduction, both of which have been related to AD in previous tissue and CSF proteomics studies.^{5,6} It is commonly hypothesized that amyloid depositions trigger a neuroinflammatory response involving several signal transduction pathways,¹⁷ which may be captured in our proteomic measurements. Another biological process that was associated with progression to dementia was the MAPK cascade, which involves many signaling pathways and has been implicated in multiple aspects of AD pathogenesis, including neuroinflammation, tau phosphorylation, and synaptic plasticity.¹⁸ Our results suggest that dysregulation of MAPK pathways may contribute to faster cognitive decline and could thus potentially serve as a therapeutic target.

It is known that higher levels of proteins associated with neuronal injury are related to faster decline (e.g., total tau, p-tau) in prodromal AD.¹⁵ While we found a group of proteins to be enriched for neuronal death, we observed that mostly lower concentrations were associated with faster progression to dementia. The functional consequence of in- or decreased CSF protein levels in general remains largely unclear, as CSF protein concentrations do not always correspond to protein concentrations in brain tissue, and can also, for example, reflect active sequestering of proteins or a dysregulation between endo- and exocytosis. It would be interesting to further investigate the relationship between longitudinal changes in CSF protein levels during disease progression, and eventually changes in tissue.

A potential limitation of our study is that we used targeted proteomics, and so we may have missed other proteins that play a role in AD pathogenesis. Still, an advantage of the Olink panels is that they are versatile, and allow developing a prognostic neurodegeneration panel that is easy to use in clinical practice. Another potential limitation is our relatively small sample size, resulting in wide confidence intervals for our analyses, and the relatively short follow-up time. Given the heterogeneity in prodromal AD, further studies are needed to replicate and validate these results in larger samples. Furthermore, it would also be interesting to study the influence of other clinical features like vascular comorbidities and cognitive profiles. A strength of our study is that our memory clinic population is well phenotyped with clinical follow-up.

In conclusion, our results provide more insight into processes involved in early AD pathophysiology and subsequent progression to dementia.

ACKNOWLEDGMENTS

This research was performed at the Amsterdam UMC Alzheimer Center, which is part of the neurodegeneration research program of Amsterdam Neuroscience (www.amsterdamresearch.org). The Alzheimer Center Amsterdam is supported by Stichting Alzheimer Nederland and Stichting VUmc fonds. The clinical database structure was developed with funding from Stichting Dioraphte. This work was supported by ZonMW Memorabel grant program (#733050824 and #73305056) and IMI EMIF-AD. The funding sources had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

CONFLICT OF INTEREST

E. Vromen received funding for the present work from ZonMW Memorabel (#733050824, support made to the institution). M. Del Campo Milán received payment from Sociedad Española de Neurología for a presentation at its symposium in 2019. P. Scheltens has received consultancy fees (paid to the institution) from AC Immune, Alkermes, Alnylam, Alzheon, Anavex, Biogen, Brainstorm Cell, Cortexyme, Denali, EIP, ImmunoBrain Checkpoint, GemVax, Genentech, Green Valley, Novartis, Novo Nordisk, PeopleBio, Renew LLC, Roche. He is PI of studies with AC Immune, CogRx, FUJI-film/Toyama, IONIS, UCB, and Vivoryon. He is a part-time employee of Life Sciences Partners Amsterdam. He

serves on the board of Brain Research Center and New Amsterdam Pharma. C. Teunissen is a member of the Innogenetics International Advisory Boards of Fujirebio/Innogenetics and Roche. She has received research reagents from ADxNeurosciences and Euroimmun. She has a collaboration contract with ADx Neurosciences and with Quanterix and has performed contract research or received grants from AC Immune, AxonNeurosciences, Biogen, Boehringer, Brainstorm Therapeutics, Celgene, CogRx, EIP Pharma, Esai, Fujirebio, Janssen prevention center, PeopleBio, Probiobdrug, Roche, Toyama, and Vivoryon. Research of C. Teunissen is supported by the European Commission (Marie Curie International Training Network, JPND), Health Holland, the Dutch Research Council (ZonMW), The Weston Brain Institute, Alzheimer Netherlands, Alzheimer Association. P. Visser received funding for the present work from IMI EMIF-AD (support made to the institution). He received grant support from IMI, Biogen ZonMW (support made to the institution), and funding from Synapse for a workshop on grant writing. B. Tijms received funding for the present work from ZonMW Memorabel (#73305056, support made to the institution). P. Visser and B. Tijms have a patent on CSF proteomic subtypes in AD (#19165795.6 or #PCT/NL2020/050216; Applicant: Stichting VUmc).

ORCID

Eleonora M. Vromen  <https://orcid.org/0000-0001-7774-5500>

REFERENCES

- Mulder C, Verwey NA, van der Flier WM, et al. Amyloid-beta(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. *Clin Chem*. 2010;56(2):248-253.
- Vos SJ, Verhey F, Frolich L, et al. Prevalence and prognosis of Alzheimer's disease at the mild cognitive impairment stage. *Brain*. 2015;138(Pt 5):1327-1338.
- Jack CR Jr, Bennett DA, Blennow K, Research Framework NIA-AA. Toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14(4):535-562.
- Pedrero-Prieto CM, Garcia-Carpintero S, Frontinan-Rubio J, et al. A comprehensive systematic review of CSF proteins and peptides that define Alzheimer's disease. *Clin Proteomics*. 2020;17:21.
- Wesenhagen KEJ, Teunissen CE, Visser PJ, Tijms BM. Cerebrospinal fluid proteomics and biological heterogeneity in Alzheimer's disease: a literature review. *Crit Rev Clin Lab Sci*. 2020;57(2):86-98.
- Whelan CD, Mattsson N, Nagle MW, et al. Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer's disease. *Acta Neuropathol Commun*. 2019;7(1):169.
- Duits FH, Brinkmalm G, Teunissen CE, et al. Synaptic proteins in CSF as potential novel biomarkers for prognosis in prodromal Alzheimer's disease. *Alzheimers Res Ther*. 2018;10(1):5.
- Simonsen AH, McGuire J, Hansson O, et al. Novel panel of cerebrospinal fluid biomarkers for the prediction of progression to Alzheimer dementia in patients with mild cognitive impairment. *Arch Neurol*. 2007;64(3):366-370.
- van der Flier WM, Pijnenburg YA, Prins N, et al. Optimizing patient care and research: the Amsterdam Dementia Cohort. *J Alzheimers Dis*. 2014;41(1):313-327.
- Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):270-279.

11. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):263-269.
12. Tijms BM, Willemsse EAJ, Zwan MD, et al. Unbiased approach to counteract upward drift in cerebrospinal fluid amyloid-beta 1-42 analysis results. *Clin Chem*. 2018;64(3):576-585.
13. Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014;9(4):e95192.
14. Olink.com. 2021. *Home - Olink*. [online] Available at: <<https://www.olink.com/>>.
15. van Rossum IA, Vos SJ, Burns L, et al. Injury markers predict time to dementia in subjects with MCI and amyloid pathology. *Neurology*. 2012;79(17):1809-1816.
16. Spellman DS, Wildsmith KR, Honigberg LA, et al. Development and evaluation of a multiplexed mass spectrometry based assay for measuring candidate peptide biomarkers in Alzheimer's Disease Neuroimaging Initiative (ADNI) CSF. *Proteomics Clin Appl*. 2015; 9(7-8):715-731.
17. Heneka MT, Golenbock DT, Latz E. Innate immunity in Alzheimer's disease. *Nat Immunol*. 2015;16(3):229-236.
18. Albert-Gasco H, Ros-Bernal F, Castillo-Gomez E, Olucha-Bordonau FE. MAP/ERK signaling in developing cognitive and emotional function and its effect on pathological and neurodegenerative processes. *Int J Mol Sci*. 2020;21(12):4471.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Vromen EM, Milán MDC, Scheltens P, Teunissen CE, Visser PJ, Tijms BM. CSF proteomic signature predicts progression to Alzheimer's disease dementia. *Alzheimer's Dement*. 2022;8:e12240. <https://doi.org/10.1002/trc2.12240>