Association of CSF GAP-43 With the Rate of Cognitive Decline and Progression to Dementia in Amyloid-Positive Individuals

Annika Öhrfelt[a*1] (PhD), Andréa L. Benedet[a1] (PhD), Nicholas J. Ashton[a,,c,d,e] (PhD), Alzheimer's Disease Neuroimaging Initiative[2,], Hlin Kvartsberg (PhD)[a,b], Manu Vandijck (PhD)[f], Michael W Weiner (Prof, MD)[g], John Q Trojanowski (Prof, MD)[h], Leslie M Shaw (Prof., PhD)[h], Henrik Zetterberg (Prof, MD)[a,e,i,j,k], and Kaj Blennow (Prof, MD)[a,b]

Affiliations:

[a]*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*

[b]*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*

[c]*Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden*

[d]*Department of Old Age Psychiatry, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom.*

[e]*NIHR Biomedical Research Centre for Mental Health and Biomedical Research Unit for Dementia at South London and Maudsley NHS Foundation, London, United Kingdom*

[f]*Fujirebio Europe nv, Ghent, Belgium*

[g]*Department of Veterans Affairs Medical Center, Center for Imaging of Neurodegenerative Diseases, San Francisco, CA, USA; Department of Radiology, University of California, San Francisco, CA, USA; Department of Medicine, University of California, San Francisco, CA, USA; Department of Psychiatry, University of California, San Francisco, CA, USA; Department of Neurology, University of California, San Francisco, CA, USA*

[h]*Department of Pathology and Laboratory Medicine, Institute on Aging, Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.*

[i]*Department of Neurodegenerative Disease, UCL Institute of Neurology, London, United Kingdom*

[j]*UK Dementia Research Institute, London, United Kingdom*

[k]*Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China*

*Corresponding author: Annika Öhrfelt, Clinical Neurochemistry Laboratory, Inst. of Neuroscience and Physiology, Dept. of Psychiatry and Neurochemistry, Sahlgrenska Academy at the University of Gothenburg, Sahlgrenska University Hospital, Mölndal, SE-431 80 Mölndal, Sweden

Tel: +46 31 343 24 06 (office), 46 31 343 0025 (secretary) Fax: +46 31 343 24 26

[1]These authors contributed equally to this work

[2]Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at

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E-mail addresses to all authors:

Annika Öhrfelt	annika.ohrfelt@neuro.gu.se
Andréa L. Benedet	andrea.benedet@gu.se
Hlin Kvartsberg	hlin.kvartsberg@neuro.gu.se
Nicholas J. Ashton	nicholas.ashton@gu.se
Manu Vandijck	manu.vandijck@fujirebio-europe.com
Michael W Weiner	Michael.Weiner@ucsf.edu
John Q Trojanowski	trojanow@upenn.edu
Leslie M Shaw	les.shaw@uphs.upenn.edu; shawlmj@mail.med.upenn.edu
Henrik Zetterberg	henrik.zetterberg@clinchem.gu.se
Kaj Blennow	kaj.blennow@neuro.gu.se

Search terms

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Glossary

aa=amino acids

Aβ=amyloid beta

AD=Alzheimer's disease

CI=cognitively impaired

CU=cognitively unimpaired

CV=coefficients of variation

FDG=fluorodeoxyglucose

GAP43=growth-associated protein 43

LTP=long-term potentiation

MCI=mild cognitive impairment

MMSE=mini-mental state examination

PBS=phosphate-buffered saline

P-tau= tau phosphorylated at threonine 181 (P-tau)

QC=quality control

ROC=receiver operating characteristic

T-Tau=total tau

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-H. Zetterberg has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Denali, Eisai, Roche, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx, and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

-K. Blennow has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. The other authors declare that they have no competing interests.

ABSTRACT

Background and objectives: To test the associations between the presynaptic growth-associated protein 43 (GAP-43) protein, quantified in cerebrospinal fluid (CSF), and biomarkers of Alzheimer's disease (AD) pathophysiology, cross-sectionally and longitudinally.

Methods: In this retrospective study, GAP-43 was measured in participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort using an in-house ELISA method, and levels were compared between groups, both cross-sectionally and longitudinally. Linear regression models tested the associations between biomarkers of AD (Aβ and tau pathologies, neurodegeneration and cognition) adjusted by age, sex and diagnosis. Linear mixed effect models (LME) evaluated how baseline GAP-43 predicts brain hypometabolism, atrophy and cognitive decline over time. Cox-proportional hazard regression models tested how GAP-43 levels and Aβ status, at baseline, increased the risk of progression to AD dementia over time.

Results: This study included 786 participants from the ADNI cohort, which were further classified in cognitively unimpaired (CU) Aβ-negative (nCU-=197); CU Aβ-positive (nCU+=55), mild cognitively impaired (MCI) Aβ-negative (nMCI-=228), MCI Aβ-positive (nMCI+=193) and AD dementia Aβ-positive (nAD=113). CSF GAP-43 levels were increased in Aβ-positive compared to Aβ-negative participants, independent of the cognitive status. In Aβ-positive participants, high baseline GAP-43 levels led to worse brain metabolic decline (P=0.01), worse brain atrophy (P=8.8x10-27) as well as worse MMSE scores (P= 0.03) over time, as compared to those with low GAP-43 levels. Similarly, Aβ-positive participants with high baseline GAP-43 had the highest risk to convert to AD dementia (hazard ratio [HR=8.56, 95% CI, 4.94-14.80, P=1.5x10-14]). Despite the significant association with Aβ pathology (η 2 Aβ PET=0.09, P Aβ PET<0.001), CSF tTau and P-Tau had a larger effect size on GAP43 than had Aβ PET (η 2 pTau-181=0.53, P pTau-181<0.001; η 2 tTau=0.59, P tTau<0.001).

Conclusions: and Classification of Evidence: This study provides Class III classification of evidence that high baseline levels of CSF GAP-43 are associated to progression in Aβ-positive individuals, with a more aggressive neurodegenerative process, faster rate of cognitive decline and increased risk of converting to dementia.

MAIN MANUSCRIPT

Introduction

Accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles (NFT) together with synaptic loss and neurodegeneration are fundamental features of the Alzheimer's disease (AD) pathophysiology. It is known that both tau and amyloid aggregation exert vulnerable effects on synapse integrity [1], whilst synapse loss and/or synapse degeneration are suggested to be much closer related to cognitive decline than the other pathological hallmarks of AD [2-5].

Even though synaptic degeneration and loss are core characteristics of the AD pathophysiological process, it is not evident how early during disease progression synaptic dysfunction appears. Synapse loss occur in AD [3, 6-9] and many synaptic proteins have been demonstrated at reduced levels in hippocampus and neocortices, regions affected by AD pathophysiology [4, 10, 11]. In recent years, cerebrospinal fluid (CSF) synaptic biomarkers, such as neurogranin, growth-associated protein 43 (GAP-43), SNAP-25 and synaptotagmin proteins [12-14 15], have shown promising results. The CSF levels of these synaptic proteins were found to be markedly increased in patients with AD and prodromal AD [12-16]. Furthermore, high levels of the post-synaptic marker neurogranin correlates with future cognitive decline in mild cognitive impaired (MCI) patients [15, 16], suggesting that synaptic biomarkers indicate the synaptic loss and degeneration that is known to occur in AD [3, 8].

Growth-associated protein 43 (GAP-43), or neuromodulin, is a presynaptic protein vastly linked to neurite outgrowth, axonal guidance, synaptic plasticity and establishment of novel memories [17-19]. Specifically in relation to AD pathology, immunohistochemistry studies have shown altered GAP-43 concentration in cortical regions and hippocampus [20-22], known brain regions impacted by Aβ plaques, NFT, neuronal and

synaptic degeneration early in AD [3, 23, 24]. CSF GAP-43 was suggested to be a promising candidate biomarker of AD [14, 25], however, studies evaluating the prognostic potential of GAP-43 to predict cognitive decline and conversion of subjects to dementia are needed.

We aimed to evaluate, with data from the multicentric Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, the cross-sectional and longitudinal associations between GAP-43 and core biomarkers of AD. In addition, we evaluated the prognostic ability of CSF GAP-43 levels to predict cognitive decline and conversion to AD dementia.

METHODS

Participants

This report uses data obtained from the ADNI database (http://adni.loni.usc.edu/), which was launched in 2004 by the National Institute on Aging, the Food and Drug Administration, private pharmaceutical companies and non-profit organizations as a highly innovative public-private partnership, led by Principal Investigator Michael W. Weiner, MD, VA Medical Center and University of California, San Francisco. Subjects have been recruited from over 50 sites across the USA and Canada (for up-to-date information, see http://adni.loni.usc.edu/) and ethical committees of all institutions have approved the study. All participants have provided informed consent. In addition, the present study was performed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline [26].

This study initially included 802 participants, ranging from clinically diagnosed cognitively unimpaired (CU), mild cognitive impairment (MCI) and AD dementia participants, which had available CSF GAP-43 measurements as well as paired baseline CSF Aβ42 and phosphorylated tau (pTau)-181 data (data accessed on June 2021). The AD subjects met criteria for probable AD according to the National Institute of Neurological and Communicative

Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [27], with a Mini-Mental State Examination (MMSE) ranging between 20 and 26 (inclusively) and Clinical Dementia. Rating (CDR) equals 1. Participants were classified as MCI if MMSE ranged between 24 and 30, CDR of 0.5 (with the memory box score being 0.5 or greater), largely intact general cognition and functional performance, and could not meet criteria for dementia according to the NINCDS-ADRDA (for further details see [28]). In addition, participants were classified according to the Aβ status, as further described, and AD dementia participants with no evidence of Aβ pathology were excluded from our analysis, leading to a final sample size of 786 participants.

CSF Biomarkers

The GAP-43 analysis was performed using an in-house ELISA method at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital (Mölndal, Sweden) by a board-certified laboratory technician blinded to clinical information as previously described [14]. All standards and control samples were analyzed in duplicate. The intermediate precision of the GAP-43 assay was determined using two quality control human CSF samples (QC 1 and QC 2), which had an intra-assay coefficient of variation (CV) of 5.5% and 11% and inter-assay CV of 6.9% and 15.6%, respectively. For this study, the first GAP-43 measurement was used to define the baseline visit in all analyses. Longitudinal GAP-43 quantifications were available for 344 participants (227 with baseline plus one follow-up visit, 116 with baseline plus two follow-up visits and 1 with baseline plus three follow-up visits).

CSF Aβ42, total tau (tTau) and pTau-181 were quantified using the fully automated Elecsys assays (Roche Diagnostics) as reported elsewhere [29]. A positive Aβ status was given to participants who had CSF pTau-181/Aβ42 ratio > 0.028 at the baseline GAP-43 visit. Only cross-sectional Aβ42, total tau (tTau) and pTau-181 data were used in our analyses.

Neuroimaging Methods

MRI and PET summary measures were downloaded from the ADNI database and scan acquisitions followed the reported protocols (http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/ and http://adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/).

Cross-sectional brain Aβ burden was estimated using [^{18}F]florbetapir PET, in which the global load is given based on the average standardized uptake value ratio (SUVR) of the precuneus, cingulate, inferior parietal, medial prefrontal, lateral temporal, and orbitofrontal cortices, and had the pons as reference region [30]. Glucose uptake was indexed by [^{18}F]Fluorodeoxyglucose (FDG) PET, and the global SUVR was the average SUVR of the bilateral angular, posterior cingulate and inferior temporal gyri, with the cerebellar vermis and the pons used as the reference regions [31]. Longitudinal FDG PET was used in this study, counting from baseline GAP-43, and 375 participants had data for more than one visit.

Brain atrophy was determined using hippocampal and whole brain volumes. Automated volume measures were performed using FreeSurfer software package [32] and were adjusted for total intracranial volume (ICV) using data from all cognitively impaired subjects as baseline, as previously described [33]. Longitudinal brain volume was used in this study, counting from baseline GAP-43, and 729 participants had data for more than one visit.

Statistical Analysis

Biomarker and demographic data were compared between groups chi-square test, for categorical variables, and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test when variables were continuous. Linear regression models (LM) tested the associations between GAP-43 concentrations and other variables at baseline, always adjusting for age and sex. Participants were also grouped according to baseline levels of GAP-43 in terciles (low,

medium, high), as well as according to baseline Aβ PET and CSF pTau-181 in quartiles (1^{st},2^{nd},3^{rd},4^{th}).

Linear mixed effect (LME) models were employed to evaluate longitudinal relationships, which always included random intercepts and were adjusted for age, sex, and baseline measures when needed. The models were fit using maximum likelihood estimation and time was set as continuous variable, counting from baseline GAP-43. First, GAP-43 progression over time was compared between categorical groups. Then, participants were grouped according to baseline GAP-43 extreme terciles (low and high) and Aβ status and biomarker longitudinal changes were assessed. These models had longitudinal FDG PET, longitudinal MMSE and longitudinal brain atrophy as outcome measures (independently), time as continuous variable, random intercept and age, sex, education and baseline measurements as covariates.

Cox-proportional hazard regression models tested the association between groups (GAP-43 extreme terciles and Aβ status) and the risk of incident AD dementia or risk of diagnosis progression. The outcome of the model was time to diagnosis, and it was adjusted for age and sex. Participants were sensored at their last follow-up visit. Hazard ratios (HR) were reported. Schoenfield residuals tested the assumption of proportional hazards and Martingale residuals assessed nonlinearity.

To facilitate comparison and interpretation of findings, LM and LME were performed using standardized variables when indicated. GAP-43 was log transformed before standardization. All statistical analyses were performed in R statistical platform v.3.6.3 [34].

Data Availability Statement

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

RESULTS

Main characteristics of the study sample

A total of 786 participants were included in the study; 197 Aβ-negative cognitively unimpaired (CU-), 55 Aβ positive cognitively unimpaired (CU+), 228 Aβ-negative mild cognitively impaired (MCI-), 193 Aβ positive mild cognitively impaired (MCI+) and 113 AD dementia (AD) participants. The average age of the population was 72.2 (± 7.2) years old, 48% were females and average years of formal education was 16.2 (± 2.6) years. Specifics about groups characteristics can be found in Table 1, where we show that CU+ (P<0.0001) and AD (P=0.02) are in average older than CU-, whilst MCI- are younger (P=0.005). MMSE scores are found lower in MCI-, MCI+ and AD groups in comparison to CU groups, as expected. In addition, AD and MCI+ subjects have a larger proportion of *APOE* ε4 carriers in comparison to CU- group. In addition, biomarkers of Aβ and tau pathologies are abnormal in Aβ positive groups as compared to CU- subjects.

Baseline levels of GAP-43 better reflects tau pathology than Aβ pathology

Cross-sectional GAP-43 levels were shown to be SIGNIFICANTLY? OR NOT? increased in Aβ positive groups as compared to CU- subjects, whilst MCI- had slightly lower levels (Figure 1A). We found no association between GAP-43 and age (P=0.25; adjusting by sex and diagnosis) but a sex effect was found, where females had higher levels than males (P=0.02; adjusting by age and diagnosis). Linear models tested the effect of Aβ PET, CSF pTau-181 and tTau on GAP-43, and, despite being all significant associations, CSF pTau-181 and tTau had a larger effect on GAP-43 than had Aβ PET ($\eta^2_{\text{Aβ PET}}$=0.09, $P_{\text{Aβ PET}}$<0.001; $\eta^2_{\text{pTau-181}}$=0.53, $P_{\text{pTau-}}$

$_{181}$<0.001; η^2_{tTau}=0.59, P_{tTau}<0.001). This relationship was clearly visualized when we compared GAP-43 levels between quartile groups (Figure 1B-C).

GAP-43 has steeper increasing levels in participants with low baseline measurements.

When evaluating longitudinal changes, we did not observe differences on GAP-43 levels between pure clinically defined or "biomarker defined" diagnostic groups over time (Figure 2A-B). However, when segregating participants based on GAP-43 terciles, we found that low baseline GAP-43 levels lead to a steeper trajectory than does high baseline GAP-43, suggesting that GAP-43 plateaus over time (Figure 2C-D).

Baseline levels of GAP-43 is associated with metabolic decline and brain atrophy over time.

GAP-43 showed no association with baseline FDG (*P*=0.57). However, when participants were grouped according to GAP-43 levels and Aβ status, higher GAP-43 was associated to worse metabolic decline over 96 months (Figure 3A-B). In addition, GAP-43 showed no associations with cross-sectional hippocampal volume (*P*=0.83) but it was associated with brain volume (*P*<0.001; adjusted by age, sex, diagnosis and education). High baseline GAP-43 was also linked to greater brain atrophy over time (Figure 3C-D).

High baseline levels of GAP-43 predict faster cognitive decline and higher risk of dementia.

Higher levels of GAP-43, cross-sectionally, were found to be associated with worse cognitive performance on the MMSE (*P*=0.01) as well as to predict worse cognitive decline over 96 months (Figure 4A-B). Corroborating these findings, the survival analysis showed that high baseline GAP-43 and positivity for Aβ pathology was the profile which showed the greatest

risk of converting to dementia (hazard ratio [HR]=8.56, 95% CI, 4.94-14.80; Figure 4C) or to clinically progress (HR=5.80, 95% CI, 3.61-9.33; Figure 4D) over the period of 6 years.

Discussion

In the present study we show that high CSF GAP-43 levels are associated with increased risk to dementia onset and are associated with faster decline in cognition. Particularly in Aβ positive individuals, a more rapid decline in cognitive performance was observed in participants with high CSF levels in contrast to participants with lower GAP-43 levels. Similarly, in the presence of amyloid pathology, high CSF GAP-43 concentrations indicated an increased risk to convert to AD dementia. In addition, baseline CSF GAP-43 predicted more metabolic decline evaluated by FDG-PET and increased brain atrophy assessed by MRI.

In the current study, we showed that baseline GAP-43 levels were increased in Aβ-positive groups as compared to CU Aβ-negative group. Our results are in agreement with most of the previous studies, reporting elevated CSF levels of GAP-43 in AD [14, 35, 36] and in MCI due to AD compared to controls [25, 35]. Interestingly, the observation of significantly higher levels of GAP-43 already in CU+ group compared to CU-, indicates that synaptic alterations related to amyloidosis may occur even before clinical symptoms are manifested [37]. We have also shown no differences in GAP-43 progression over time between investigated groups. Since GAP-43 levels reflect synaptic loss/degeneration, which is the main correlate to cognitive decline [3, 6], monitoring of changes of the trajectories of GAP-43 over time might be useful to test the efficacy of drugs in intervention studies for AD. When participants were classified according to their baseline levels of GAP-43 into low, medium and high groups, we observed that having low levels of baseline GAP-43 led to progressive higher concentrations of the biomarker

longitudinally as compared to no changes in its levels when the baseline was already high, suggesting that the biomarker plateaus over time.

When evaluating the association between GAP-43 and core AD biomarkers cross-sectionally, linear models showed that CSF tTau and pTau-181 had a larger effect on GAP-43 than had Aβ PET, which suggests that CSF GAP-43 are more tightly associated with tau pathology and neurodegeneration than it is with Aβ pathology. In line with our results, previous studies showed a strong association between GAP-43 and tau pathology at a cross-sectional level, whilst only a weak or a lack of correlation of CSF GAP-43 and Aβ were found [14, 25]. In fact, as the dual main functions of GAP-43 are related to regeneration of axons and synapses [38], CSF levels of GAP-43 may reflect both degeneration of axons and decline of presynaptic function[21].

Cross-sectional GAP-43 levels were also related to longitudinal cognitive performance. High baseline levels of the biomarker predicted worse cognitive decline, indexed by MMSE, over time in both Aβ positive and negative groups when these were compared to participants with initial low levels of GAP-43. Corroborating these findings, Aβ-positive individuals with high baseline GAP-43 had the highest risk to progress clinically and to convert to dementia. In alignment with those findings, the levels of neurogranin, the post-synaptic counterpart of GAP-43, were previously found associated to the severity of cognitive decline in AD [15, 39, 40]. These calmodulin binding proteins appear to be inevitable for neuronal transmission and synaptic plasticity[41, 42], thereby their changes might reflect early signs of cognitive decline.

We have shown that high baseline GAP-43 levels were associated to greater brain atrophy and worse metabolic decline over time, as proxied by longitudinal measures of brain volume and FDG-PET. As these biomarkers indicate neurodegeneration, these findings further support the

concept that synaptic abnormalities precede cell dysfunction and death, as previously suggested [43-45]. There might be subtle differences among the biomarkers reflecting neurodegeneration (MRI, FDG-PET, CSF-tau) [44]. In line with our results, studies based on CSF biomarkers have shown that synaptic alterations precede and/or parallels neurodegeneration in preclinical AD [46, 47].

I DON'T THINK YOU ARE REALLY DISSCUSSING THE RESULTS IN THE DISCUSSION, YOU ARE MOSTLY REPEATING THE RESULTS, WITH A FEW COMMENTS. ID LIKE TO SEE MORE DISCUSSION ABOUT WHAT IS NEW HERE, AND WHAT IS THE MEANING OF ALL OF THIS. WHAT HAVE WE LEARNED? DOES THIS NEW MARKER ADD SOMETHING TO OTHER CSF BIOMARKERS?. IS THIS SOMETHING TO BE USED IN TREATMENT TRIALS

Limitations

There are some limitations of our study. Although model were adjusted for them, demographic characteristics differed between groups.

Conclusions

High baseline levels of GAP-43 were mostly linked to increased tau pathology as well as associated with future decline in brain metabolism, progressive brain atrophy, cognitive decline, and higher risk to progress to dementia. Altogether, these results support the framework that synaptic changes stand in between AD pathological changes and future neurodegeneration and cognitive symptoms. Furthermore, findings point to GAP-43 as a potential marker of clinical progression particularly in subjects with Aβ pathology.

Appendix 1. AUTHORS

Name	Location	Contribution
Annika Öhrfelt, PhD	University of Gothenburg, Mölndal, Swe	Interpreted the data and prepared the manuscript.
Andréa Lessa Benedet, PhD	University of Gothenburg, Mölndal, Swe	Analyzed the data, performed the statistical analyses and prepared the manuscript.
Hlin Kvartsberg, PhD	University of Gothenburg, Mölndal, Swe, and Sahlgrenska University Hospital, Mölndal, Swe	Drafted the manuscript for the intellectual content.
Nicholas James Ashton, PhD	University of Gothenburg, Mölndal, Swe, Sahlgrenska University Hospital, Mölndal, Swe, University of Gothenburg, Gothenburg, Sweden, King's College London, UK, and NHS Foundation, London, UK	Drafted the manuscript for the intellectual content.
Michael W Weiner (Prof, MD)	University of California, San Francisco, CA, USA	Drafted the manuscript for the intellectual content.
John Q Trojanowski (Prof, MD)	University of Pennsylvania School of Medicine, Philadelphia, PA, USA	Drafted the manuscript for the intellectual content.
Leslie M Shaw (Prof., PhD)	University of Pennsylvania School of Medicine, Philadelphia, PA, USA	Drafted the manuscript for the intellectual content.
Henrik Zetterberg, MD, Prof	University of Gothenburg, Mölndal, Swe, Sahlgrenska University Hospital, Mölndal, Swe, UCL Institute of Neurology, London, UK, UK Dementia Research Institute, London, UK and Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China	Conceptualized the study and drafted the manuscript for the intellectual content.
Kaj Blennow, MD, Prof	University of Gothenburg, Mölndal, Swe, and Sahlgrenska University Hospital, Mölndal, Swe	Designed and conceptualized the study, analyzed the data and prepared the manuscript.

Appendix 2 CO-INVESTIGATORS

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc. edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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Figure Legends

Figure 1. Cross-sectional GAP-43. Distribution of CSF GAP-43 concentrations across groups, showing Aβ negative groups with lower levels of GAP-43 as compared to Aβ positive groups (A; all Aβ positive groups are significantly different from Aβ negative groups, $P<0.0001$). GAP-43 levels were also compared between Aβ PET (B; 3rd and 4th quartiles are significantly higher than 1st and 2nd quartiles, $P<0.001$) and CSF pTau-181 (C; all groups are significantly different from each other, $P<0.0001$) quartile groups. *P* values of group comparisons were corrected for multiple comparisons.

Figure 2. Longitudinal progression of GAP-43. Linear mixed effect models tested the evolution of CSF GAP-43 over time between groups. In A, we found no difference between the slopes of the groups (shaded areas represent confidence intervals (CI)), which is also represented in B by forest plots. When participants were grouped according to baseline GAP-43 levels (tercile groups), high GAP-43 at baseline showed no changes over time, which was significantly different from steeper biomarker progression when baseline GAP-43 levels were low ($^{***}P=3\text{x}10^{-5}$), as shown in plots C and D (shaded areas represent CI).

Figure 3. GAP-43 levels predicting longitudinal metabolic decline and brain atrophy. Linear mixed effect models first compared FDG changes between GAP-43 and Aβ groups over time. Plots A and B show that all groups have faster FDG decline in comparison with Aβ negative (Aβ-) participants with low baseline GAP-43 ($^{***}P_{\text{Low GAP-43 Aβ+}}= 1.1\text{x}10^{-4}$; $^{***}P_{\text{High GAP-43 Aβ-}}= 7.5\text{x}10^{-5}$; $^{***}P_{\text{High GAP-43 Aβ+}}= 2.2\text{x}10^{-16}$). Results also showed that, in Aβ positive (Aβ+) subjects, high GAP-43 levels led to worse FDG hypometabolism over time as compared to low GAP-43 levels ($^{*}P=0.01$; shaded areas represent confidence intervals (CI)). Similar models were also performed to compare changes in brain volume over time. As shown in plots C and D, rates of brain atrophy were greater in participants with low GAP-43 and Aβ+ ($^{***}P=1.2\text{x}10^{-5}$), high GAP-43 and Aβ- ($^{**}P=0.008$) and high GAP-43 and Aβ+ groups ($^{**}P=0.001$) in contrast with low GAP-43 and Aβ- group. In addition, in Aβ+ individuals, longitudinal brain atrophy was worse in those who had high GAP-43 at baseline in comparison to those with low GAP-43 ($^{***}P=8.8\text{x}10^{-27}$).

Figure 4. GAP-43 levels suggesting cognitive decline. Linear mixed effect models compared MMSE changes between GAP-43 and Aβ groups over time (A and B). Aβ positive (Aβ+) groups had worse decline in MMSE scores when compared with participants Aβ negative (Aβ-) with low baseline GAP-43 levels ($^{***}P_{\text{Low GAP-43 Aβ+}}$= 1.8x10^{-22}; $^{***}P_{\text{High GAP-43 Aβ+}}$= 3.3x10^{-46}). In Aβ+ participants, high GAP-43 at baseline also indicated worse MMSE scores over time as comparated to those with low GAP-43 ($^{*}P$= 0.03). Cox-proportional hazard model (adjusted by age, sex and education) showing that, in comparison to low GAP-43 Aβ- group, low levels of baseline GAP-43 and Aβ+ are associated with an increased risk to convert to AD dementia (hazard ratio [HR]=4.17, 95% CI, 2.04-8.49, P=8,3x10^{-5}), which the highest risk was found for high GAP-43 and Aβ+ group (HR=8.56, 95% CI, 4.94-14.80, P=1.5x10^{-14}), as evidenced by the Kaplan-Meier curves (C). When comparing Aβ+ groups, high GAP-43 had highest conversion rate (HR=2.05, 95% CI, 1.13-3.07, P=0.01). Similarly, when evaluating rates of diagnosis progression, as shown by Kaplan-Meier curves (D), in comparison to low GAP-43 Aβ- group, low levels of baseline GAP-43 and Aβ+ are associated with an increased risk to progress clinically (HR=3.67, 95% CI, 1.98-6.78, P=3,3x10^{-5}), which the highest risk was found for high GAP-43 and Aβ+ group (HR=5.80, 95% CI, 3.61-9.33, P=3,8x10^{-13}).

Table 1. Demographic and biomarker summary information of the sample.

	CU- (n=197)	**MCI-** (n=228)	**CU+** (n=55)	**MCI+** (n=193)	**AD** (n=113)
Age, years	72.0 (5.78)	70.1 (7.61)**	75.9 (5.61)***	72.8 (6.93)	73.9 (8.39)*
Female, n (%)	104 (52)	110 (48)	37 (67) [a]	81 (44)[a]	50 (44)
Education, years	16.8 (2.49)	16.2 (2.58)*	16.0 (2.33)***	16.0 (2.72)***	15.6 (2.68)***
***APOE*-ε4 carriers, n (%)**	44 (22)	63 (27)	29 (52)***	143 (74)***	82 (72)***
MMSE	29.0 (1.16)	28.5 (1.47)***	28.9 (1.20)	27.4 (1.85)***	23.0 (2.05)***
CSF pTau-181/Aβ42	0.01 (0.004)	0.01 (0.005)	0.04 (0.01)***	0.05 (0.02)***	0.06 (0.03)***
CSF pTau-181, pg/mL	18.9 (6.26)	18.0 (6.11)	31.7 (11.7)***	36.3 (15.0)***	38.7 (16.1)***
CSF tTau, pg/mL	215.0 (72.0)	204.2 (65.0)	317.8 (110.8)***	358.7 (135.0)***	387.7 (156.4)***
CSF GAP-43, pg/mL	4570 (2200)	4040 (2000)*	6460 (3600)***	6420 (3120)***	6430 (3230)***
Aβ PET, SUVR	1.06 (0.11)	1.05 (0.12)	1.36 (0.20)***	1.40 (0.17)***	1.44 (0.18)***
FDG PET, SUVR	1.32 (0.10)	1.30 (0.11)*	1.21 (0.09)	1.21 (0.13)***	1.04 (0.13)***
Hippocampal vol., mm^3	7633 (783)	7368 (1085)***	7391 (692)	6688 (1011)***	5950 (801)***
Whole brain vol., mm^3	1070000 (54800)	1070000 (62200)*	1050000 (44600)	1050000 (58600)***	1010000 (57000)***

Abbreviations: Aβ42, amyloid-β 42; AD, Alzheimer's Disease; CSF, cerebrospinal fluid; CU-, Aβ-negative cognitively unimpaired; CU+, Aβ-positive cognitively unimpaired; FDG, [18F]fluorodeoxyglucose, GAP-43, Growth-associated protein 43, MCI+, Aβ-positive mild cognitive impairment; MMSE, Mini-Mental State Examination; p-tau181, tau phosphorylated at threonine 181; t-tau, total tau.

Data shown as mean (SD) or n (%), as appropriate. One-way ANCOVA was used to compare age, education years and MMSE between groups (adjusting by sex) and Pearson's chi-square to compare sex and *APOE*-ε4 frequencies between groups. Imaging and fluid biomarkers were compared with a one-way ANCOVA adjusted by age and sex. Aβ status for group definition was based on CSF pTau-181/Aβ42 ratio. Hippocampal and whole brain volumes are adjusted by intra-cranial volume.

$^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; for these CU- was the reference group.

$^{a}P<0.05$ between these groups.