Published Ahead of Print on December 22, 2021 as 10.1212/WNL.0000000000013228

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The Official Journal of the American Academy of Neurology

AMERICAN ACADEMY OF NEUROLOGY®

Neurology Publish Ahead of Print
DOI: 10.1212/WNL.0000000000013228

Association of CSF Aβ38 Levels With Risk of Alzheimer Disease Related Decline

Author(s):
Nicholas Cullen, BS[1]; Shorena Janelidze, PhD[1]; Sebastian Palmqvist, MD, PhD[1, 2]; Erik Stomrud, MD, PhD[1, 2]; Niklas Mattsson-Carlgren, MD, PhD[1, 3, 4]; Oskar Hansson, MD, PhD[1, 2]

Corresponding Author:
Nicholas Cullen
nicholas.cullen@med.lu.se

Affiliation Information for All Authors: 1. Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Faculty of Medicine, Lund University, Lund, Sweden; 2. Memory Clinic, Skåne University Hospital, Malmö, Sweden;3. Department of Neurology, Skåne University Hospital, Lund, Sweden; 4. Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden

Contributions:
Nicholas Cullen: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Shorena Janelidze: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data; Additional contributions: SJ performed biomarker measurements.
Sebastian Palmqvist: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data; Additional contributions: S.P. managed clinical evaluations and logistics.
Erik Stomrud: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data; Additional contributions: E.S. managed clinical evaluations and logistics.
Niklas Mattsson-Carlgren: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data; Additional contributions: N.M.-C. supervised the work.
Oskar Hansson: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data; Additional contributions: O.H. supervised the work.

Publication History: This manuscript was pre-published in www.medrxiv.org/content/10.1101/2021.01.31.21250702v1

Number of characters in title: 120

Abstract Word count: 269

Word count of main text: 4132

References: 43

Figures: 3

Tables: 3

Supplemental: STROBE Guidelines

Statistical Analysis performed by: Nicholas Cullen, Lund University, BS

Search Terms: [26] Alzheimer's disease, [36] Cognitive aging

Acknowledgements: The authors thank the participants of the BioFINDER and ADNI studies and their families.

Study Funding: Work at the authors research center was supported by the Swedish Research Council (2016-00906), the Knut and Alice Wallenberg foundation (2017-0383), the Marianne and Marcus Wallenberg foundation (2015.0125), the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson s disease) at Lund University, the Swedish Alzheimer Foundation (AF-939932), the Swedish Brain Foundation (FO2019-0326), The Parkinson foundation of Sweden (1280/20), the Skåne University Hospital Foundation (2020-O000028), Regionalt Forskningsstöd (2020-0314) and the Swedish federal government under the ALF agreement (2018-Projekt0279). Doses of 18F-flutemetamol injection were sponsored by GE Healthcare.

Disclosures: N.C. Cullen, S. Janelidze, S. Palmqvist, E. Stomrud, N. Mattsson-Carlgren report no disclosures relevant to the manuscript. O. Hansson has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Alzpath, Biogen, Cerveau and Roche.

Abstract

Objective: Experimental studies suggest that the balance between short and long Aβ species might modulate the toxic effects of Aβ in Alzheimer's disease (AD) but clinical evidence is lacking. We studied whether Aβ38 levels in cerebrospinal fluid (CSF) relate to risk of AD dementia and cognitive decline.

Methods: CSF Aβ38 levels were measured in 656 individuals across two clinical cohorts – the Swedish BioFINDER study and the Alzheimer's Disease Neuroimaging Initiative (ADNI). Cox regression models were used to evaluate the association between baseline Aβ38 levels and risk of AD dementia in AD-biomarker positive individuals (AD+; determined by CSF P-tau/Aβ42 ratio) with subjective cognitive decline (SCD) or mild cognitive impairment (MCI). Linear mixed effects models were used to evaluate the association between baseline Aβ38 levels and cognitive decline as measured by the Mini-Mental State Examination (MMSE) in AD+ participants with SCD, MCI or AD dementia.

Results: In the BioFINDER cohort, high Aβ38 levels were associated with slower decline in MMSE (β = 0.30 points / sd., P = 0.001) and with lower risk of conversion to AD dementia (HR = 0.83 per sd., P = 0.03). In the ADNI cohort, higher Aβ38 levels were associated with less decline in MMSE (β = 0.27, P = 0.01), but not risk of conversion to AD dementia (P = 0.66). Aβ38 levels in both cohorts were significantly associated with both cognitive and clinical outcomes when further adjusted for CSF P-tau or CSF Aβ42 levels.

Interpretation: Higher CSF Aβ38 levels are associated with lower risk of AD-related changes in two independent clinical cohorts. These findings suggest that γ-secretase modulators could be effective as disease-altering therapy.

Keywords: Aβ38, amyloid, Aβ peptides, amyloid cascade, γ-secretase modulators

Introduction

After many promising clinical trials of Alzheimer's disease (AD), only recently has a treatment been shown to potentially delay cognitive decline in a Phase III clinical trial [1–3]. In any case, effects on cognition by available treatments for AD are modest at best [4]. Currently, the most widely accepted explanation for AD pathogenesis is the amyloid cascade hypothesis, which proposes that AD is primarily initiated by accumulation of the beta-amyloid (Aβ) peptide into senile plaques, sequentially followed by the accumulation of misfolded tau protein into tangles, neuronal loss, and cognitive decline along with loss of independence in activities of daily living (ADL) [5].

An expected consequence of the amyloid cascade hypothesis is that modulating the production of Aβ levels in the brain should prevent downstream effects of this pathology and thereby slow the disease course. It is precisely this mechanism which has been the target of recent AD therapies, although recent clinical trials have demonstrated moderate effect on disease progression as measured by cognitive tests [6–9]. Resolving the disagreement between overwhelming evidence speaking for amyloid's role as disease driver and the previous failure of most (but not all) anti-amyloid therapies is therefore a major open question in the AD research field.

One proposed explanation for this failure is that the canonical view of amyloid accumulation may be oversimplified, particularly as it relates to the 42-amino acid long peptide (Aβ42) which has been the primary focus of fluid biomarker studies. Increasing evidence suggests that the relative abundance of different Aβ isoforms, especially those shorter than Aβ42, may play a more decisive role in AD pathogenesis than previously thought [10,11]. For instance, many presenilin mutations known to cause a familial form of AD do not directly result in higher Aβ42 levels in the brain, but rather disturb the relationship between

Aβ42 and shorter Aβ species through a loss-of-function mechanism [12–14]. A role for shorter Aβ species in AD development could explain why targeting of amyloid aggregates expressed primarily by Aβ42 levels is not sufficient to halt the trajectory of AD. This is especially relevant due to renewed interest in targeting diverse mechanisms of amyloid toxicity within the pharmaceutical industry, such as γ-secretase modulators (GSMs) [15,16]. Investigating the association between shorter Aβ peptides and AD-related changes is therefore important for understanding amyloid accumulation, particularly as it relates to disease-altering therapies.

In the present study, we took a clinical approach to this question by measuring Aβ38 levels in cerebrospinal fluid (CSF) and characterizing them with regards to risk of developing AD dementia as well as cognitive decline. Our analysis was performed in two large, independent cohorts comprised of individuals spread broadly across the AD spectrum. Our primary aim was to understand whether CSF Aβ38 levels relate to AD-relevant clinical outcomes and thereby shed more light on the complex relationship between the amyloid protein and AD.

Methods

Study design and participants

Participants recruited for the Swedish BioFINDER study were enrolled consecutively between 2010 and 2014 (clinical trial no. NCT03174938). Participants consisted of consecutively included non-demented patients with mild cognitive symptoms referred to participating memory clinics as previously described [17]. The inclusion criteria were (i) referred to the memory clinic due to cognitive symptoms experienced by the patient and/or informant (note, these symptoms were not necessarily memory complaints, but could also be

executive, visuospatial, language, praxis, or psychomotor complaints), (ii) between 60 and 80 years old, (iii) baseline MMSE score between 24 and 30 points, (iv) did not fulfill criteria for any dementia, and (v) fluent in Swedish. The primary exclusion criteria were (i) significant systemic illness or organ failure, (ii) ongoing alcohol or substance misuse, (iii) refusing lumbar puncture or neuropsychological assessment, and (iv) cognitive symptoms which could be directly explained by another condition or disease. At baseline, patients were categorized as having either subjective cognitive decline (SCD) or mild cognitive impairment (MCI) based on an extensive neuropsychological battery examining verbal, episodic memory, visuospatial ability and attention/executive domains. Further, patients with AD who fulfilled the NIA-AA criteria for probable AD were included in the present analysis [19]. All participants were enrolled consecutively after being referred to a memory clinic and had follow-up visits every year. All relevant ethical committees approved the BioFINDER study, and all study participants gave written informed consent.

Additional data was analyzed from participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) study, which was launched in 2003 as a public-private partnership. Participants in the ADNI study have been recruited from more than 50 locations across the United States and Canada. Inclusion and exclusion criteria for ADNI have been described in detail previously [20]. Briefly, all ADNI participants were between the ages of 55 and 90 years, had completed at least six years of education, were fluent in Spanish or English, and had no significant neurologic disease other than AD. Regional ethical committees of all institutions approved the ADNI study and all study participants gave written informed consent. ADNI data was downloaded from http://adni.loni.usc.edu on 2020/03/01.

The present analysis included only AD-biomarker positive ("biomarker-positive") participants, determined based on an abnormal CSF P-tau/Aβ42 ratio for which cutoffs have been previously established in both cohorts [21]. Moreover, all participants had a diagnosis of

SCD, MCI, or AD and were only included in the present analysis if they had available baseline values for age, sex, education, CSF Aβ38, CSF Aβ42, CSF P-tau, MMSE, and at least one follow-up visit where MMSE was measured.

Predictors and outcomes

Demographic characteristics including age, sex, and education were collected from all participants. Various biomarkers of amyloid processing or pathology – Aβ38, Aβ40, Aβ42, and amyloid precursor protein (sAPP; available in BioFINDER only) – along with tau phosphorylated at threonine 181 (P-tau) were measured in CSF at baseline in all participants. All biomarkers were natural log-transformed prior to analysis in order to obtain a more normal distribution of biomarker values. In BioFINDER, Aβ38, Aβ40, Aβ42, P-tau, and sAPP levels were measured using a standard ELISA assay (Euroimmun, Lubeck, Germany). In ADNI, Aβ38, Aβ40, and Aβ42 levels in CSF were measured using a 2D-UPLC tandem mass spectrometry method at the University of Pennsylvania (first made publicly available in February 2020). CSF P-tau levels in ADNI were measured using the Elecsys platform (Roche, Basel Switzerland).

The primary outcome was longitudinal change in cognition as measured by the Mini-Mental State Examination (MMSE) scale. MMSE is a cognitive test which is highly relevant to cognitive changes in AD and is often used as a basis for making a clinical diagnosis or inclusion into clinical trials [22]. The secondary clinical outcome was the Preclinical Alzheimer's Cognitive Composite (PACC) while was developed specifically to identify early cognitive changes in individuals without dementia [23]. The modified PACC score used in the current study was made up of MMSE, delayed word recall from the Alzheimer's Disease Assessment Scale (ADAS)-Cognitive Subscale (weighted double to reflect the emphasis on

memory tests in the original PACC), animal fluency, and trail-making B tests. The primary clinical outcome was development of AD dementia at any time during longitudinal follow-up. Clinical status was evaluated and recorded at each follow-up visit by a physician experienced in dementia disorders. A diagnosis of AD required abnormal amyloid accumulation as evidenced by CSF or PET levels along with consensus evaluation of Clinical Dementia Rating (CDR) and the Function Activities Questionnaire (FAQ).

Statistical Analysis

Linear mixed effects (LME) modelling was used to assess the relationship between continuous Aβ38 levels (adjusted for age, sex, and education) and the primary study outcome of longitudinal change in MMSE. Additional LME models were fit which also included covariate adjustment for CSF Aβ42 or P-tau levels. LME models had random intercepts and slopes with an interaction term between time and Aβ38 levels and an interaction term between time and Aβ42 or P-tau levels for models which also included those biomarkers. Additional analysis of longitudinal cognition was performed in which a demographic-adjusted model including the Aβ38/Aβ40 ratio was directly compared to a model using the Aβ42/Aβ40 ratio instead.

Cox regression modelling was used to assess the association between continuous Aβ38 levels (adjusted for age, sex, and education) and conversion to AD dementia during longitudinal follow-up. Additional cox regression models were fit which also included covariate adjustment for either CSF Aβ42 or P-tau levels. All participants were right-censored (i.e., the last follow-up visit was considered as either the latest visit if the participant was never diagnosed with AD dementia or the visit when diagnosis of AD dementia occurred) and the proportionality of hazards assumption was assessed using Schoenfeld residuals.

All biomarkers and continuous demographic variables were standardized prior to all model fitting in order to increase comparability of standardized model coefficients across cohorts. The analysis of longitudinal cognition included all study participants (SCD, MCI, AD) while the analysis of longitudinal risk for AD dementia included only participants who did not already have AD dementia (SCD, MCI).

All code was written in the R programming language (v4.0.0) and all significance tests were two-sided with alpha = 0.05 as significance threshold.

Research Questions

Our primary research question was whether there was an association between CSF Aβ38 and AD-related outcomes evaluated longitudinally in participants with SCD, MCI, or AD who had abnormal AD biomarker signatures. A secondary question was how the association between CSF Aβ38 and AD-related outcomes was modulated by further controlling for other Aβ-related biomarkers, P-tau, and APOE status.

Standard Protocol Approvals, Registrations, and Patient Consents

All participants gave written informed consent to participate in the BioFINDER study as approved by the ethical committee of Lund University, Sweden. All participants gave written informed consent to participate in the ADNI study as approved by the ethical committees of all participating sites. All methods were carried out in accordance with the approved guidelines.

Data availability

All relevant source data from the present manuscript along with anonymized data from the BioFINDER study will be shared by request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with EU legislation on the general data protection regulation and decisions by the Ethical Review Board of Sweden and Region Skåne, which should be regulated in a material transfer agreement. The code used for statistical analyses is available at a public repository.

Results

Cohort characteristics

In the BioFINDER cohort (see Table 1), we included 338 biomarker-positive (defined by an elevated CSF Aβ42/P-tau ratio; representing 55.8% of 605 eligible participants in the study) participants classified as either SCD (n = 54), MCI (n = 150), or AD (n = 134). The average age was 72.5 ± 6.8 years, 52.1% of participants were female, and average education was 11.0 ± 3.5 years. The average follow-up time was 4.0 ± 1.6 years, with 91.1% of participants having at least a two-year visit and 65.3% of participants having at least a four-year visit.

In the ADNI cohort (see Table 2), we included 318 biomarker-positive participants (47.8% of 665 eligible participants in the study) classified as either SCD (n = 17), MCI (n = 192), or AD (n = 109). The average age was 73.1 ± 7.4 years, 54.7% of participants were female, and average education was 15.9 ± 2.8 years. The average follow-up time was 3.7 ± 2.5 years, with 73.1% of participants having at least a two-year visit and 49.5% of participants having at least a four-year visit.

We compared demographic variables between cohorts and found no significant difference in participant age (P = 0.28) or in the percentage of male/female participants (P = 0.49). However, participants from the ADNI cohort had significantly higher educational attainment than participants from the BioFINDER cohort (difference = 4.85 years, P < 0.001).

We tested the relationship between biomarkers in both cohorts independently (Figure 1) and found that CSF Aβ38 was significantly correlated with Aβ42 (r = 0.44, P < 0.0001 in BioFINDER; r = 0.54, P < 0.0001 in ADNI) and with P-tau (r = 0.37, P < 0.0001 in BioFINDER; r = 0.53, P < 0.0001 in ADNI). Looking across diagnosis in the BioFINDER cohort, the association between CSF Aβ38 and CSF Aβ42 in the BioFINDER cohort was highest in the AD group (r = 0.59) compared to in MCI (r = 0.37) and SCD (r = 0.41) groups. Meanwhile, in the ADNI cohort the association between CSF Aβ38 and CSF Aβ42 was highest in SCD (r = 0.70) and AD (r = 0.66) compared to MCI (r = 0.47). And while our primary analysis included only biomarker-positive individuals we found that this association was higher in biomarker-negative individuals (r = 0.63). Moreover, CSF Aβ38 levels did not significantly differ by APOE status in the BioFINDER cohort (P = 0.20 for zero ε4 copies versus one ε4 copies; P = 0.15 for zero ε4 versus two ε4 copies).

Association with longitudinal decline in cognition

In the BioFINDER cohort, higher CSF Aβ38 levels adjusted for demographics (age, sex, and education) were associated with less decline in MMSE over time (β = 0.30 points / year per std. of biomarker change, P = 0.001). Higher Aβ38 levels adjusted for Aβ42 levels were also associated with less decline in MMSE over time (β = 0.25, P = 0.03); Aβ42 in the same model was not significantly associated with MMSE change (P = 0.57). Higher Aβ38 levels with additional adjustment for P-tau were associated with less decline in MMSE over time (β

= 0.76, P < 0.0001); P-tau in the same model was also significantly associated with MMSE change (β = -0.94, P < 0.0001). These results are displayed graphically in Figure 2.

In the ADNI cohort, higher Aβ38 levels adjusted for demographics (age, sex, and education) were associated with less decline in MMSE over time (β = 0.27, P = 0.01). Higher Aβ38 levels with additional adjustment for Aβ42 were also associated with less decline in MMSE over time (β = 0.32, P = 0.03); Aβ42 in the same model was not significantly associated with MMSE change (P = 0.61). Finally, higher Aβ38 levels with additional adjustment for P-tau were associated with less decline in MMSE over time (β = 0.90, P < 0.0001); P-tau in the same model was also significantly associated with MMSE change (β = -0.95, P < 0.0001). These results are displayed graphically in Figure 2.

Using the PACC scale in the ADNI cohort showed that higher Aβ38 levels were associated with longitudinal change in PACC adjusted only for covariates (β = 0.37, P = 0.016) and adjusted for covariates and CSF P-tau (β = 1.35, P < 0.0001), but not when additionally adjusted for CSF Aβ42 (β = 0.34, P = 0.11).

Moreover, using the Aβ38/Aβ40 and Aβ42/Aβ40 ratios directly as predictors showed that neither ratio measure was associated with longitudinal change in MMSE for the BioFINDER cohort (P = 0.34 and P = 0.55, respectively). However, there was a significant association for Aβ38/Aβ40 (β = 0.30, P = 0.0056) but not Aβ42/Aβ40 (P = 0.39) for the same outcome in the ADNI cohort. These results were qualitatively similar when the composite PACC score was used as cognitive outcome.

Finally, we tested the association of CSF Aβ38 with the outcomes of interest while controlling for additional variables of interest. When controlling directly for diagnostic status with longitudinal cognition as outcome, CSF Aβ38 levels adjusted for age, sex, education,

and diagnostic status was significantly associated with MMSE (β = 0.30, P = 0.001) but not PACC (β = -0.06, P = 0.31) in the BioFINDER cohort, and was significantly associated with both MMSE (β = 0.22, P = 0.036) and PACC (β = 0.30, P = 0.045) in the ADNI cohort. To note, the overall model R^2 increased greatly when including diagnostic status as outcome while the standardized regression coefficient for Aβ38 was generally smaller. Moreover, there was no significant change in association between Aβ38 levels and longitudinal cognition for any model when controlling directly for number of APOE ε4 copies.

Association with risk of AD dementia

In the BioFINDER cohort, higher Aβ38 levels adjusted for demographics were associated with lower risk of AD dementia (HR = 0.83 higher odds per std. of biomarker change, 95% CI [0.71, 0.98], P = 0.03), while higher Aβ38 levels additionally adjusted for Aβ42 trended towards an association with lower risk of conversion (HR = 0.85 [0.69, 1.05], P = 0.12), and higher Aβ38 levels additionally adjusted for P-tau were strongly associated with lower risk of conversion (HR = 0.56 [0.46, 0.69], P < 0.0001). These results are displayed graphically in Figure 3.

In the ADNI cohort, there was not a significant association with conversion to AD dementia when Aβ38 levels were adjusted only for demographics (HR = 0.96 [0.79, 1.16], P = 0.66), and there was not a significant association when Aβ38 was adjusted for demographics and Aβ42 (HR = 0.94 [0.73, 1.21], P = 0.62). Still, higher Aβ38 levels adjusted for demographics and P-tau were strongly associated with lower risk of conversion to AD dementia (HR = 0.55 [0.43, 0.71], P < 0.0001). These results are displayed graphically in Figure 3.

Analysis of other Aβ biomarkers

We performed the same analyses above and in the same groups but using CSF Aβ40 as the variable of interest instead of CSF Aβ38. In BioFINDER, higher CSF Aβ40 levels adjusted only for demographics were associated with less decline in MMSE (β = 0.30 points / year per std. of biomarker change, P = 0.01; not significantly different than the effect size of Aβ38, P = 0.48). Aβ40 levels were also associated with less decline in MMSE when adjusted additionally for CSF Aβ42 (β = 0.27, P = 0.04) and CSF P-tau (β = 0.72, P < 0.0001). In ADNI, higher Aβ40 levels adjusted for only demographics were not associated with higher change in MMSE (β = 0.21, P = 0.051), nor when also adjusted for CSF Aβ42 (β = -0.09, P = 0.15), but were significant when adjusted for CSF P-tau (β = 0.84, P < 0.0001). To note, the standardized effect sizes for Aβ40 in the ADNI cohort were smaller in magnitude for all models than those that instead included Aβ38.

With regards to conversion to AD dementia in the BioFINDER cohort, CSF Aβ40 levels adjusted only for demographics were weakly associated with conversion to AD dementia (HR = 0.84 lower odds per std. of biomarker change, P = 0.048). As with Aβ38, higher Aβ40 levels were not associated with conversion to AD dementia when adjusted further for Aβ42 (HR = 0.86, P = 0.21), but did have a significant association when adjusted further for P-tau (HR = 0.61, P < 0.0001). The standardized effect size for Aβ40 was smaller for all modes in the BioFINDER cohort compared to the same models with Aβ38. In ADNI, CSF Aβ40 levels were not associated with conversion to AD dementia when adjusted only for demographics (HR = 1.03, P = 0.78) or additionally for Aβ42 (HR = 1.08, P = 0.61), but were significantly associated when adjusted further for P-tau (HR = 0.60, P = 0.0003). The standardized effect size for significant Aβ40 models in the ADNI cohort was again smaller than the corresponding models that included Aβ38 instead.

A similar analysis of CSF sAPP levels (available only in BioFINDER) showed no association with change in MMSE (β = -0.01, P = 0.94) or conversion to AD dementia (HR = 0.94, P = 0.65).

Discussion

There is a great need in the AD research field to explain why overwhelming evidence points to amyloid being the key driver of AD pathogenesis while anti-amyloid therapies have had up until the present day only rather modest effect on disease progression in late-stage clinical trials. One proposed explanation to recent trial results has been to question inherent factors of the trials themselves – e.g., inclusion criteria, choice of endpoint, too late treatment initiation, or statistical power [24]. However, more recent AD trials have included stringent biomarker inclusion criteria, sophisticated composite clinical endpoints, and large numbers of participants [7,8,25].

Assuming then that these trial failures are due to biological factors, another response has been to instead question the entire amyloid cascade hypothesis by suggesting that amyloid accumulation may be an indirect effect rather than primary cause of AD [26]. However, existence of early-onset familial AD caused by mutations in the *APP*, *PSEN1*, and *PSEN2* genes – all part of the Aβ processing machinery – suggests that the true explanation for the lack of anti-amyloid therapies' immediate success should still retain the integrity of the amyloid cascade hypothesis [27]. One possible mechanism could be that there is a more complex interaction between different Aβ peptides than previously appreciated, which would explain why targeting of Aβ42 alone may not be sufficient to halt the disease progression. Recent evidence suggests that lower levels of shorter Aβ peptide levels or lower ratio of shorter to longer Aβ peptides could be an important factor in Aβ toxicity [11,15,16].

Our results in the current study support this hypothesis from a clinical perspective, as we demonstrated that higher CSF Aβ38 levels are associated with less cognitive decline and lower risk of developing AD dementia in individuals who are biomarker-positive (see Table 3 for summary of evidence). The adjustment of our statistical models for core AD biomarkers (Aβ42, P-tau) despite using them as inclusion criteria into the present analysis reflects our attempt to handle the idea that binary cutoffs must necessarily be used in patient workflows, but for prognostic modelling it is best to use continuous biomarker values. Our finding that higher CSF Aβ38 levels are protective even in the presence of significant AD pathology in the brain (we only included biomarker-positive individuals) should motivate further studies to understand the molecular underpinning of a potential protective mechanism from Aβ38, and possibly even Aβ40. For instance, it is unclear if Aβ38 levels modulate the development of tau pathology in individuals who already reached thresholds for Aβ positivity pathology. Interestingly, we also found that the Aβ38/Aβ40 ratio was a stronger predictor of longitudinal cognitive decline than Aβ42/Aβ40 in individuals with AD pathology.

The validation of our findings in two independent cohorts with differing demographic profiles – ADNI participants have high educational attainment on average and are primarily typical amnestic AD cases, while the BioFINDER cohort is more heterogenous in demographical and diagnostic makeup – adds validity to our results. Still, the validation is strengthened by the finding that standardized effect sizes of Aβ38 were similar across cohorts (e.g., $\beta = 0.30$ for Aβ38 in BioFINDER and $\beta = 0.27$ for Aβ38 in ADNI, for longitudinal MMSE as outcome). Importantly, the effect sizes for Aβ38 in the statistical models were generally stronger than those for Aβ40 or sAPP – indicating a specific effect of Aβ38 rather than simply an effect of total Aβ production or APP cleavage. Taken together, then, it is unlikely that our findings could be due to systematic changes related to CSF collection,

volume, or measurement. Our results were also largely replicated across two different cognitive scales in the ADNI cohort, indicating that choice of cognitive measure should not affect results significantly.

These findings are of particular importance due to the renewed interest in γ-secretase modulators (GSMs), a class of drugs which reduces Aβ42 production while maintaining total Aβ production by blocking cleavage of amyloid precursor protein (APP) at specific γ-secretase cleavage sites [28,29]. In comparison to previously tested γ-secretase inhibitors (GSIs), which had untenable off-target effects in past clinical trials, GSMs do not alter total Aβ production and thus do not compromise the broader biological role of γ-secretase [29,30]. Previous studies of GSMs have shown that the Aβ38 peptide does not exhibit any toxicity *in vivo* (nor does it accumulate into plaques following overexpression in mice) and can even protect against Aβ42-associated dysfunction [15]. However, while the Aβ42/Aβ40 ratio has been widely implicated in both clinical studies of AD and animal studies of GSMs (primarily as a proxy for brain Aβ build-up), few clinical studies prior to ours have investigated Aβ38 levels [31–33].

Unfortunately, no GSM compound has yet been brought to a phase III clinical trial. However, a review of the literature reveals that hindrances of GSMs in early-phase trials relate largely to poor penetrance into the brain or economic concern [34,35]. Nonetheless, work on GSM compounds within the AD field remains active both with regards to pre-clinical animal studies [36], *in vitro* studies demonstrating significant effects on amyloid processing and accumulation in relevant disease models [37], and computational studies investigating potential modulator binding sites or mechanisms of action leading to identification of clinical candidates [38,39]. Due to the accumulating evidence of GSM compounds affecting amyloid processing, it is likely that these drugs will be targeted towards individuals with familial AD

and it is therefore important to closely follow results from ongoing studies in such populations such as the DIAN-TU prevention trial [40].

To summarize, our results suggest that further investigations should be undertaken to understand whether increasing the relative levels of shorter Aβ peptides such as Aβ38 is in fact an effective strategy to treat AD [41,42]. Importantly, we did not test whether CSF Aβ38 has added clinical value over well-established biomarkers of amyloid, tau, and neurodegeneration. Instead, we provided clinical evidence here that higher Aβ38 levels are in fact associated with lower risk of AD-related changes, which may support the use of GSMs as an approach to altering AD progression. Still, our understanding of the interaction between the different Aβ peptides is still lacking. Additionally, we restricted our analysis to AD biomarker-positive individuals for multiple reasons. Firstly, rates of cognitive decline and AD dementia are low among AD biomarker-negative individuals [43]. Secondly, biomarker-positive individuals are the target for nearly all disease-altering therapies in AD and we therefore aimed to understand whether Aβ38 levels modulate cognitive decline within this highly relevant population.

The present study was focused only on individuals with abnormal biomarker pathology, since those without abnormal biomarker pathology are highly likely to remain stable within the time scales of our study (2 – 6 years) both from a cognitive and clinical standpoint. Changes in shorter amyloid peptides in healthy, elderly individuals who may be relevant for longer term preventative AD trials is thus outside the scope of the current study and is a subject for further investigation.

Figure Captions

Figure 1: Distribution of CSF Aβ38 levels across diagnostic groups and cohorts and their association with CSF Aβ42 and CSF P-tau

This figure shows how CSF Aβ38 levels are distributed across diagnostic groups (A) and how CSF Aβ38 levels relate to CSF Aβ42 (B) and CSF P-tau (C) levels in the BioFINDER (A.a, B.a, C.a) and ADNI (A.b, B.b, C.b) cohorts. Association between biomarkers was tested using Pearson correlation. Datapoints in the scatter plots (B, C) are colored according the same scheme in the boxplots (A): red = AD, green = MCI, blue = SCD.

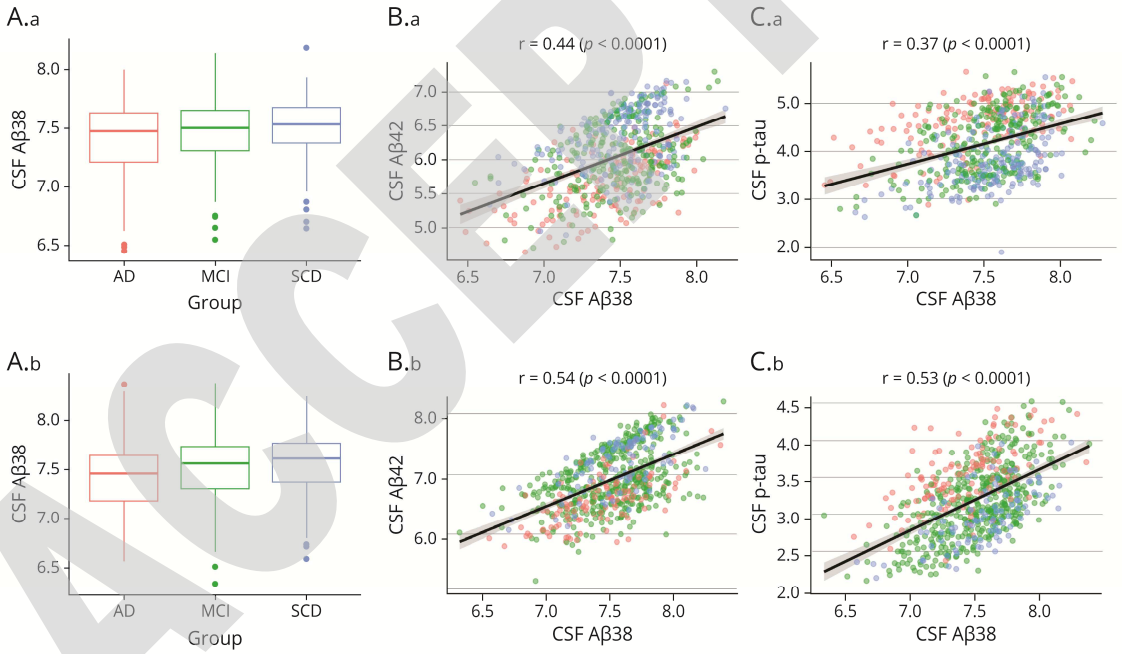


Figure 2: Association between CSF Aβ38 and longitudinal cognition across cohorts

This figure displays results from linear mixed effects analysis in the BioFINDER and ADNI cohorts to investigate the association between longitudinal MMSE and Aβ38 alone, Aβ38 adjusted for Aβ42, and Aβ38 adjusted for P-tau levels. All models were additionally adjusted for age, sex, and education. Coefficients are displayed both for the effect of Aβ38 on baseline MMSE ("baseline" in the figure) and on change in MMSE over time ("slope" in the figure).

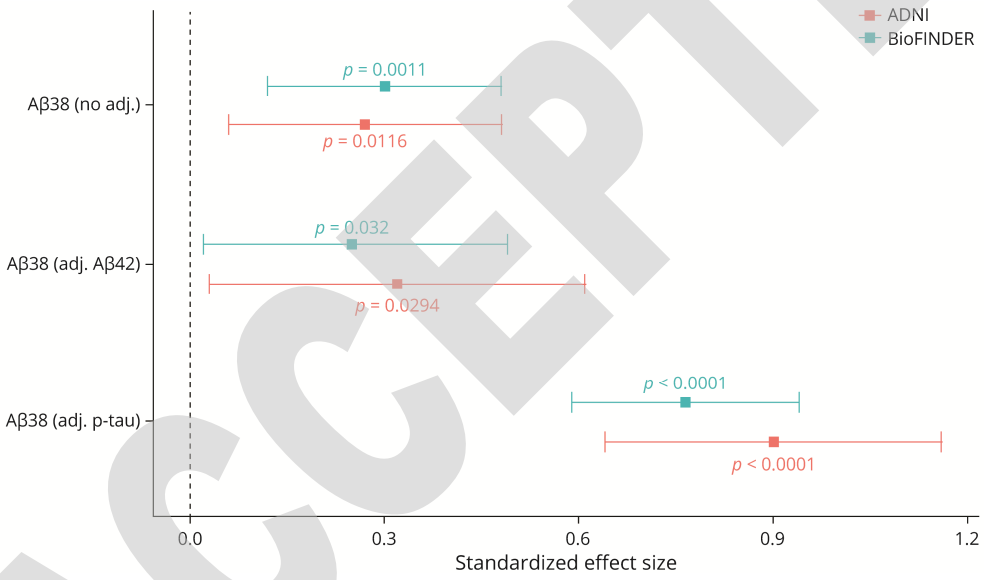
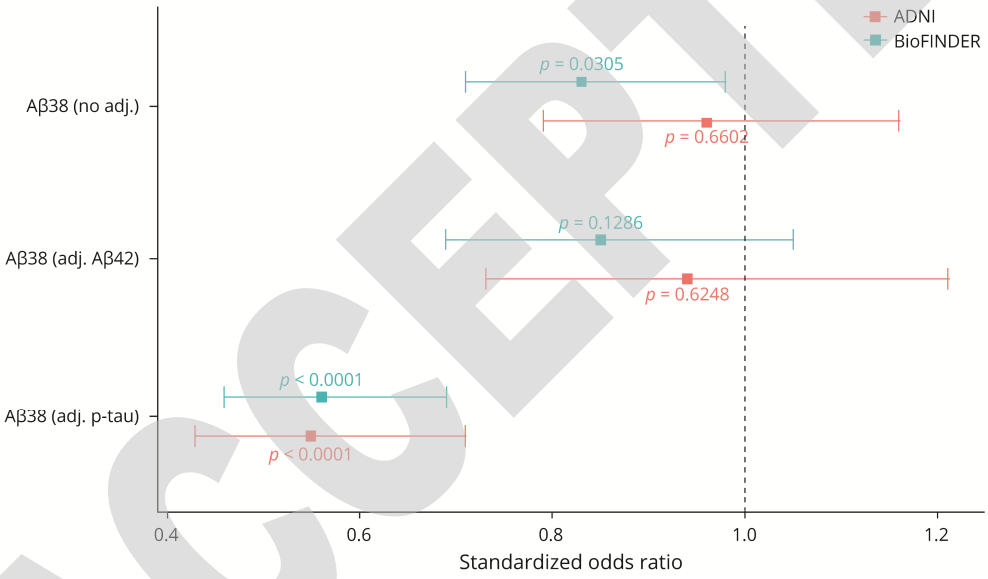


Figure 3: Association between CSF Aβ38 and clinical conversion across cohorts

This figure displays results from Cox regression analysis in the BioFINDER and ADNI cohorts to investigate the association between risk of developing AD dementia and Aβ38 alone, Aβ38 adjusted for Aβ42, and Aβ38 adjusted for P-tau levels. All models were additionally adjusted for age, sex, and education. Coefficients represents the change in odds of converting to AD dementia for each standard deviation increase in Aβ38 levels.



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Tables

Table 1: Cohort characteristics – BioFINDER

	SCD	MCI	AD	p
n	54	150	134	
Age	71.48 (5.63)	71.96 (4.90)	73.36 (8.61)	0.144
Sex, Female (%)	30 (55.6)	81 (54.0)	51 (38.1)	0.013
Education	12.19 (4.03)	11.18 (3.21)	10.30 (3.34)	0.002
MMSE (baseline)	28.02 (1.63)	26.77 (1.73)	21.44 (3.80)	<0.001
CSF Aβ38 (baseline)	7.56 (0.24)	7.51 (0.26)	7.43 (0.32)	0.006
CSF Aβ40 (baseline)	8.67 (0.32)	8.60 (0.35)	8.51 (0.43)	0.017
CSF Aβ42 (baseline)	5.82 (0.33)	5.73 (0.37)	5.63 (0.42)	0.007
CSF P-tau (baseline	4.54 (0.37)	4.62 (0.42)	4.74 (0.41)	0.005
Converted to AD dementia (%)	25 (46.3)	118 (78.7)	0 (0.0)	<0.001
Time under risk of AD dementia	4.06 (1.65)	2.69 (1.55)	NA (NA)	<0.001

This table summarizes cohort characteristics and includes the combination (i.e. union) of participants for which both longitudinal MMSE and conversion to AD were analyzed as outcomes. All continuous values are reported as mean (std. dev.). All participants were biomarker-positive for AD pathology as determined by an elevated CSF P-tau/Aβ42 ratio. SCD: subjective cognitive decline, MCI: mild cognitive impairment; AD: Alzheimer's disease.

Table 2: Cohort characteristics – ADNI

	SCD	MCI	AD	p
n	17	192	109	
Age	73.96 (5.25)	72.56 (6.95)	73.94 (8.34)	0.263
Sex, Female (%)	13 (76.5)	81 (42.2)	50 (45.9)	0.024
Education	16.65 (2.67)	15.95 (2.76)	15.55 (2.72)	0.224
MMSE (baseline)	29.29 (0.85)	27.45 (1.82)	23.04 (2.03)	<0.001
CSF Aβ38 (baseline)	7.58 (0.30)	7.56 (0.30)	7.42 (0.33)	0.001
CSF Aβ40 (baseline)	9.03 (0.28)	9.02 (0.28)	8.90 (0.32)	0.005
CSF Aβ42 (baseline)	6.66 (0.31)	6.56 (0.32)	6.48 (0.35)	0.042
CSF P-tau (baseline	3.50 (0.35)	3.53 (0.38)	3.61 (0.38)	0.164
Converted to AD dementia (%)	0 (0.0)	102 (53.1)	0 (0.0)	<0.001
Time under risk of AD dementia	3.50 (1.88)	2.84 (2.11)	NA (NA)	0.213

This table summarizes cohort characteristics and includes the combination (i.e. union) of participants for which both longitudinal MMSE and conversion to AD were analyzed as outcomes. All participants were biomarker-positive for AD pathology as determined by an elevated CSF P-tau/Aβ42 ratio. SCD: subjective cognitive decline, MCI: mild cognitive impairment; AD: Alzheimer's disease.

Table 3: Qualitative summary of results

Outcome	Aβ38		Aβ38 (+ Aβ42)		Aβ38 (+ P-tau)	
	BioFINDER	ADNI	BioFINDER	ADNI	BioFINDER	ADNI
Baseline MMSE	***	***	*	*	***	***
Change in MMSE	***	*	*	*	***	***
Conversion to AD	*	NS	NS	NS	***	***

This table summarizes evidence for the association of Aβ38 levels with AD-related changes in the BioFINDER and ADNI cohorts. Asterisks in the table represent a significant association between the biomarker model (top row) and the outcome (left-most column) in the given cohort (second-to-top row) based on tests from linear mixed effects modelling (for baseline MMSE and change in MMSE) or Cox regression modelling (for conversion to AD). All models were adjusted for age, sex, and education. NS = Not Significant; * = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.0005$.

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Neurology published online December 22, 2021
DOI 10.1212/WNL.0000000000013228