Cerebrospinal fluid levels of fatty acid–binding protein 3 are associated with likelihood of amyloidopathy in cognitively healthy individuals

Kunal Dhiman1,2,3 | Victor L. Villemagne4,5,6 | Christopher Fowler7 |
Pierrick Bourgeat8 | Qiao-Xin Li7 | Steven Collins6,7 | Christopher C. Rowe5,6 |
Colin L. Masters7 | David Ames9,10 | Kaj Blennow11,12 | Henrik Zetterberg11,12,13,14,15 |
Ralph N. Martins3,16,17,18,19,20 | Veer Gupta1,3

1 IMPACT – The Institute for Mental and Physical Health and Clinical Translation, School of Medicine, Deakin University, Geelong, Victoria, Australia
2 Western Health Partnership, School of Nursing and Midwifery (Centre for Quality and Patient Safety Research in the Institute of Health Transformation), Faculty of Health, Deakin University, Melbourne, Victoria, Australia
3 School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia
4 Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
5 Department of Molecular Imaging & Therapy and Centre for PET, Austin Health, Heidelberg, Victoria, Australia
6 Department of Medicine, The University of Melbourne, Melbourne, Victoria, Australia
7 The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia
8 Australian e-Health Research Centre, CSIRO Health and Biosecurity, Brisbane, Queensland, Australia
9 National Ageing Research Institute, Parkville, Victoria, Australia
10 Academic Unit for Psychiatry of Old age, St. George’s Hospital, The University of Melbourne, Melbourne, Victoria, Australia
11 Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
12 Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Gothenburg, Sweden
13 Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London, UK
14 UK Dementia Research Institute at UCL, London, UK
15 Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China
16 Australian Alzheimer’s Research Foundation, Ralph and Patricia Sarich Neuroscience Research Institute, Nedlands, Western Australia, Australia
17 Department of Biomedical Sciences, Macquarie University, Sydney, New South Wales, Australia
18 School of Psychiatry and Clinical Neurosciences, University of Western Australia, Perth, Western Australia, Australia
19 KaRa Institute of Neurological Diseases, Sydney, New South Wales, Australia
20 Co-operative Research Centre for Mental Health, Carlton, Victoria, Australia

Abstract
Introduction: Fatty acid–binding protein 3 (FABP3) is a biomarker of neuronal membrane disruption, associated with lipid dyshomeostasis—a notable Alzheimer’s disease (AD) pathophysiological change. We assessed the association of cerebrospinal fluid
Methods: FABP3 levels were measured in CSF samples of cognitively healthy participants, > 60 years of age (n = 142), from the Australian Imaging, Biomarkers & Lifestyle Flagship Study of Ageing (AIBL).

Results: FABP3 levels were positively associated with baseline brain amyloid beta (Aβ) load as measured by standardized uptake value ratio (SUVR, standardized β = 0.22, P = .009) and predicted the change in brain Aβ load (standardized β = 0.32, P = .004). Higher levels of CSF FABP3 (above median) were associated with a likelihood of amyloidopathy (odds ratio [OR] 2.28, 95% confidence interval [CI] 1.12 to 4.65, P = .023).

Discussion: These results support inclusion of CSF FABP3 as a biomarker in risk-prediction models of AD.

KEYWORDS
Alzheimer’s disease, amyloid, early diagnosis, risk prediction, screening

1 | BACKGROUND

Evidence of high brain amyloid beta (Aβ) or amyloidopathy, ascertained via positron emission tomography (PET) or reduced levels of cerebrospinal fluid (CSF) Aβ42, is a key criterion for the identification of preclinical Alzheimer’s disease (AD)—suggested by National Institute on Aging–Alzheimer’s Association (NIA-AA) criteria 2011 and the International Working Group-2 (IWG-2) criteria 2014 and 2016. This criterion has been used in several studies to identify cases of preclinical AD. The recently proposed research framework by NIA-AA recommends the use of biomarker guided ATN classification (A: Aβ, T: tau, N: neurodegeneration) to identify cognitively healthy individuals who have preclinical AD or preclinical AD pathological change. Cognitively healthy individuals with a biomarker profile, A+T+/N± (preclinical AD) and A+T−N− (preclinical AD pathological change) fall within the AD spectrum.

Given that neurodegenerative changes contribute to the development of AD, accompanied by amyloidopathy (increased Aβ PET), it is worthwhile to identify the dynamics of brain amyloidopathy in cognitive healthy individuals via changes in pathophysiological biomarkers specific to different aspects of neurodegeneration, such as axonopathy, neuronal membrane disruption, and perturbed Ca2+ homeostasis. Moreover, such biomarkers could quantify the effect of modifiable risk factors on the risk of developing preclinical AD. CSF fatty acid–binding protein 3 (FABP3) or heart type fatty acid–binding protein (H-FABP) is a biomarker of neuronal membrane disruption, associated with lipid dyshomeostasis—a notable AD pathophysiological change. CSF levels of this protein are elevated in AD and predict disease progression in patients with mild cognitive impairment (MCI). Evidence indicates the association of FABP3 with risk factors associated with AD—traumatic brain injury (TBI) and cardiovascular risk factors. In a recent study by Lagerstedt et al., blood levels of FABP3 have been shown to improve the predictive outcome in patients with TBI. In addition, elevated blood levels of FABP3 are associated with higher cardiovascular risk factors, and can predict cardiovascular outcomes in patients with stable coronary heart disease. Therefore, assessment of CSF or plasma levels of FABP3, and understanding their association with brain amyloidopathy (central AD pathological change) and its potential to indicate likelihood of amyloidopathy, can help determine the risk of AD among vulnerable individuals exposed to such risks—TBI and cardiovascular risk factors. Therefore, FABP3 could be used as one of the biomarkers in building biomarker-based risk-prediction models for dementia.

Herein we aimed to assess the association of CSF FABP3 levels with brain amyloidopathy and its potential to indicate the likelihood or risk of amyloidopathy. We measured CSF FABP3 levels in CSF samples of cognitively healthy participants from the Australian Imaging, Biomarkers & Lifestyle Flagship Study of Ageing (AIBL) and evaluated the association of FABP3 CSF levels with brain amyloidosis and likelihood of amyloidopathy using positron emission tomography PET Aβ imaging. The influence of apolipoprotein E (APOE) genotype, sex, and age in driving these associations was also assessed.

2 | METHODS

2.1 | Participants

This study included cognitively healthy participants (n = 142) from AIBL who gave consent for CSF collection (collected between 2009 and 2016 at either of the two study centers (Melbourne, VIC and Perth, WA) and underwent PET imaging at baseline corresponding to CSF collection. Participants were classified as cognitively healthy based on performance on neuropsychological and cognitive tests (e.g.,
RESEARCH IN CONTEXT

1. **Systematic Review**: Fatty acid-binding protein 3 (FABP3) is a biomarker of neuronal membrane disruption—a notable pathophysiological change in Alzheimer’s disease (AD). Evidence indicates the association of FABP3 with AD risk factors. Therefore, assessment of CSF levels of FABP3, and understanding their association with brain amyloidosis, could help in determining the likelihood of amyloidopathy in cognitively healthy individuals and making an early diagnosis.

2. **Interpretations**: Our findings indicate that CSF FABP3 levels are positively associated with baseline brain amyloid beta (Aβ) load, predict the change in brain Aβ load, and are associated with a likelihood of amyloidopathy.

3. **Future Directions**: Findings support the inclusion of FABP3 in diagnostic models to predict risk of AD/screen preclinical AD. Furthermore, we aim to assess the utility of CSF FABP3 to predict disease onset in cognitively healthy individuals and progression in individuals with mild cognitive impairment.

---

Sample collection and biomarker analyses

Sample collection involved lumbar puncture (LP), as per the Alzheimer’s Biomarkers Standardization Initiative protocol. Participants’ MMSE scores ranged from 24 to 30 and had a Clinical Dementia Rating (CDR) of 0. Exclusion criteria included heavy alcohol consumption (exceeding two standard drinks per day for women and four per day for men), past serious head injury, history of non-AD dementia, significant current depression (15-item Geriatric Depression Scale [GDS-15] score > 5), schizophrenia, bipolar disorder, epilepsy, amnesia, Parkinson disease, cancer (other than basal cell skin carcinoma) within the last 2 years, history of stroke, uncontrolled diabetes, lack of fluency in the English language, and withdrawal of consent. Poor performance on cognitive tests due to current medical illness, medical history (as above), or medication use was another criterion for exclusion.

The study was approved by the Human Research Ethics Committees of Edith Cowan University, Austin Health and St. Vincent’s Health and Hollywood Private Hospital. Information on participants’ baseline characteristics (sex, age, and presence of APOE ɛ4) was collected as part of cohort characterization. Aβ positivity (Aβ+) was determined using PET-derived standardized uptake value ratio (SUVR), commensurate with neocortical Aβ burden (described in the next section).

2.2 | Brain Aβ imaging

Neocortical Aβ burden (brain Aβ load) was assessed for all participants (n = 142) at baseline, and for 91 participants at follow-up, via PET using different Aβ tracers, [11C]Pittsburgh compound B ([11C-PiB]), [18F]-florbetapir, and [18F]-flutemetamol as described previously. The PET image were analyzed using the CapAIBL software. The average SUVR, computed as the area-weighted mean SUVR of different cortical regions (frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions), was used as a quantitative measure of neocortical Aβ burden. The SUVR generated with F-18 tracers were linearly transformed into PiB-like SUVR units called the Before the Centiloid Kernel Transformation (BeCKeT), to place all SUVR on a continuous scale, and Aβ positivity (Aβ+) was determined using a SUVR/BeCKeT cutoff value of 1.4. The time interval between baseline (corresponding to CSF collection) and follow-up PET imaging ranged from 1.2 to 3.5 years (average 1.7 years).

2.3 | Sample collection and biomarker analyses

Sample collection involved lumbar puncture (LP), as per the Alzheimer’s Biomarkers Standardization Initiative protocol. Samples were stored at −80°C, following centrifugation within 2 hours, at 2000 × g for 10 minutes and making aliquots into polypropylene tubes (0.5 mL). Samples went through one freeze-thaw cycle to aliquot the samples further.

Concentrations of CSF Aβ42, total tau (t-tau), and phosphorylated tau (p-tau) were measured using enzyme-linked immunosorbent assays (ELISAs): INNOTEST β-AMYLOID (Aβ42), INNOTEST hTAU Ag (t-tau), and INNOTEST PHOSPHO-TAU (p-tau 181P) (Fujirebio, Ghent, Belgium), at the National Dementia Diagnostics Laboratory (NDDL), Florey Institute, The University of Melbourne.

CSF FABP3 concentrations were quantified on the meso scale discovery (MSD) platform using electrochemiluminescence on the SECTOR Imager 2400A, with Human FABP3 Kits (Meso Scale Diagnostics, USA) according to the manufacturer’s protocol, at the laboratory of School of Medical and Health Sciences, Edith Cowan University. The assay uses detection antibodies (goat polyclonal) conjugated with electrochemiluminescent label (MSD SULFO-TAG), and assay plates are pre-coated with capture antibodies (mouse mAb) on an electrode surface. The assay has an average lower limit of detection of 0.103 ng/mL. A pooled control CSF was run to check for interplate variation. The percentage coefficient of variance (CV) between duplicates was <10% (average 2%) and between plates was 10%.

2.4 | Statistical analyses

Statistical analyses were conducted using IBM SPSS version 27 (for Microsoft Windows). Cross-sectional differences in mean values of continuous variables were assessed using analysis of covariance (ANCOVA) and t-tests. Group comparisons for categorical variables were made using chi-square tests. Correlation of CSF FABP3 with age was evaluated through Pearson rho correlation coefficient. In addition, using the median age (73 years) as a cutoff, participants were dichotomized into two groups—(1) ≤73 years and (2) >73 years—to further assess influence of age on CSF FABP3 levels by comparing
CSF levels among the two groups. Linear regression analyses were conducted to assess the effect of baseline levels of biomarkers (CSF FABP3, t-tau, and p-tau), and other variables (age, sex, and presence of APOE ε4) on baseline brain Aβ load (measured via SUVR) and change in Aβ load at follow-up. Associations between CSF biomarkers and SUVR were assessed by adjusting for covariates, ages, sex, and APOE genotype. A natural log transformation was applied to SUVR to meet the assumptions of linear regression. Participants were dichotomized into groups using median value (50th percentile) of CSF FABP3 concentration as a cutoff (75 ng/mL), as well as the value corresponding to the best sensitivity and specificity (Aβ+ vs Aβ−) as a cutoff (2.85 ng/mL). Cutoff was determined using a receiver-operating characteristic (ROC) curve analysis by keeping sensitivity (61%) and specificity (62%) approximately equal. Participants were designated as having high CSF FABP3 concentration (CSF FABP3+), if the concentration was more than the median or ROC cutoff; otherwise they were designated as CSF FABP3−. Logistic regression analyses were used to test the effect of elevated levels of FABP3 (as a categorical variable) on the likelihood of preclinical AD (identified by Aβ load as measured by SUVR/BeCkTeT, transformed to natural logarithm) was assessed via linear regression analyses. Results are summarized in Table 2. No association was noted for sex (male vs female) and age and SUVR (P = 0.167 for sex and P = 0.068 for age). The presence of APOE ε4 allele was positively associated with baseline SUVR/BeCkTeT (standardized β = 0.26, P = 0.002). Associations between CSF biomarkers (FABP3, t-tau, and p-tau) and baseline SUVR/BeCkTeT were assessed after controlling for covariates (age, sex, and APOE ε4 presence). CSF FABP3 levels were positively associated with baseline brain Aβ load as measured by SUVR/BeCkTeT, transformed to natural logarithm (standardized β = 0.22, P = 0.009), and accounted for 16% variability in baseline Aβ load. CSF measures (t-tau and p-tau) were rescaled to the same measurement unit (ng/mL) as that of CSF FABP3. CSF t-tau accounted for the maximum variability in baseline SUVR/BeCkTeT (23%) and was the stronger predictor of baseline brain Aβ load (standardized β = 0.35, p < 0.001).

3 | RESULTS

3.1 | Association of CSF FABP3 with age, sex, and APOE ε4 genotype

Participants’ ages varied between 61 and 88 years. The potential association of CSF FABP3 with age was assessed via correlational analysis. Significant correlation was noted between CSF FABP3 levels and age (r = 0.276, P < 0.001). Participants were dichotomized based on the median value of age (73 years). CSF FABP3 levels (mean levels) were significantly higher in the group with age >73 years as compared to the group with age ≤ 73 years (P = 0.006; Table 1). These results reflect the age-dependent increase in CSF FABP3 levels. A comparison of CSF FABP3 levels among APOE ε4 carriers versus non-carriers indicated no difference in CSF levels between the two groups (P = 0.410; Table 1). On the other hand, a comparison of CSF FABP3 levels between the male and female participants indicated higher CSF levels to be associated with the male sex (P = 0.005; Table 1).

3.2 | Association with brain amyloidosis—Baseline SUVR

The association of CSF FABP3 levels, demographic variables (age, sex, and APOE ε4), and core CSF biomarkers of tau pathology and neurodegeneration (CSF t-tau and t-tau) with brain Aβ load (measured by SUVR/BeCkTeT, transformed to natural logarithm) was assessed via linear regression analyses. Results are summarized in Table 2. No association was noted for sex (male vs female) and age and SUVR (P = 0.167 for sex and P = 0.068 for age). The presence of APOE ε4 allele was positively associated with baseline SUVR/BeCkTeT (standardized β = 0.26, P = 0.002). Associations between CSF biomarkers (FABP3, t-tau, and p-tau) and baseline SUVR/BeCkTeT were assessed after controlling for covariates (age, sex, and APOE ε4 presence). CSF FABP3 levels were positively associated with baseline brain Aβ load as measured by SUVR/BeCkTeT, transformed to natural logarithm (standardized β = 0.22, P = 0.009), and accounted for 16% variability in baseline Aβ load. CSF measures (t-tau and p-tau) were rescaled to the same measurement unit (ng/mL) as that of CSF FABP3. CSF t-tau accounted for the maximum variability in baseline SUVR/BeCkTeT (23%) and was the stronger predictor of baseline brain Aβ load (standardized β = 0.35, p < 0.001).

3.3 | Prediction of change in brain Aβ load

Regression analyses were carried out to test the utility of CSF FABP3 including the core CSF biomarkers to predict change in brain Aβ load (difference in baseline SUVR/BeCkTeT and follow-up SUVR/BeCkTeT, log transformed). Results are summarized in Table 2. Because the time interval between PET scans varied among individuals, analyses were
TABLE 2  Association of demographic variables and baseline CSF measures with baseline brain A\textbeta{}, and of CSF measures with change in brain A\textbeta{} as measured by SUVR

| Variable | Association with baseline SUVR/BeCKe\textalpha{} (n = 142) | Prediction of change in SUVR/BeCKe\textalpha{} (\Delta SUVR/BeCKe\textalpha{}: n = 91) |
|----------|----------------------------------------------------------|----------------------------------------------------------------------------------|
|          | \(\beta\) (SE) | Standardized \(\beta\) | \(R^2\) | \(P\) value | \(\beta\) (SE) | Standardized \(\beta\) | \(R^2\) | \(P\) value |
| Sex (male vs female) | 0.06 (0.04) | 0.13 | 0.01 | .167 | 0.70 (0.23) | 0.31 | 0.19 | .003 |
| Age at LP in years | 0.01 (0.00) | 0.15 | .026 | .668 | 3.94 (1.01) | 0.32 | 0.20 | <.001 |
| APOE 4 allele (present vs not present) | 0.14 (0.04) | 0.26 | 0.07 | .002 | 0.05 (0.02) | 0.22 | 0.16 | .009 |
| CSF t-tau ng/mL (n = 141) | 0.70 (0.16) | 0.35 | 0.23 | <.001 | 0.07 (0.02) | 0.32 | 0.18 | .004 |
| CSF p-tau ng/mL (n = 141) | 3.94 (1.01) | 0.32 | 0.20 | .001 | 3.99 (1.35) | 0.30 | 0.18 | .004 |
| CSF FABP3 ng/mL (n = 142) | 0.05 (0.02) | 0.22 | 0.16 | .009 | 0.07 (0.02) | 0.32 | 0.18 | .004 |

Note: Association of baseline CSF measures with baseline SUVR was assessed after controlling for covariates age, sex, and presence of APOE 4. SUVR and \(\Delta\)SUVR values were transformed to natural logarithm. CSF t-tau and p-tau were rescaled to ng/mL.

Abbreviations: APOE, apolipoprotein E; A\textbeta{}, amyloid beta; CSF, cerebrospinal fluid; FABP3, fatty acid–binding protein 3; LP, lumber puncture; p-tau, phosphorylated tau; SUVR, standardized uptake value ratio; t-tau, total tau.

TABLE 3  Likelihood of preclinical AD (A\textbeta{} positivity) as a function of higher levels of CSF FABP3 using (a) median value as cutoff and (b) using value corresponding to best sensitivity and specificity (A\textbeta{}+ vs A\textbeta{}–)

| A) Median value as cutoff (2.75 ng/mL) | Parameters | Odds ratio (OR) | 95% CI | \(P\) value |
|----------------------------------------|------------|----------------|--------|-------------|
| Model 1 | High SF FABP3 (FABP3+) | 2.28 | 1.12–4.65 | .023 |
| Model 2 | High SF FABP3 (FABP3+) controlled for age, sex, and presence of APOE 4 | 2.29 | 1.03–5.11 | .042 |
| Model 3 | High SF FABP3 (FABP3+) controlled for presence of APOE 4 | 2.58 | 1.20–5.51 | .015 |
| Model 4 | Interaction of high SF FABP3 (FABP3+) and presence of APOE 4 | 3.15 | 1.12–8.89 | .030 |

| B) Value corresponding to best sensitivity and specificity (A\textbeta{}+ vs A\textbeta{}–) as a cutoff (2.85 ng/mL)* | Parameters | Odds ratio (OR) | 95% CI | \(P\) value |
|------------------------------------------------|------------|----------------|--------|-------------|
| Model 1 | High SF FABP3 (FABP3+) | 2.62 | 1.28–5.33 | .008 |
| Model 2 | High SF FABP3 (FABP3+) controlled for age, sex, and presence of APOE 4 | 2.86 | 1.28–6.42 | .011 |
| Model 3 | High SF FABP3 (FABP3+) controlled for presence of APOE 4 | 3.11 | 1.44–6.73 | .004 |
| Model 4 | Interaction of high SF FABP3 (FABP3+) and presence of APOE 4 | 3.26 | 1.09–9.79 | .035 |

Note: *Cutoff was determined by keeping sensitivity (61%) and specificity (62%) approximately equal.

Abbreviations: APOE, apolipoprotein E; A\textbeta{}, amyloid beta; CSF, cerebrospinal fluid; FABP3, fatty acid–binding protein 3.

controlled for the time interval between PET scans, in addition to age, sex, and APOE 4 presence. CSF FABP3 levels predicted the change in brain A\textbeta{} load or the change in SUVR/BeCKe\textalpha{} (standardized \(\beta\) = 0.32, \(P = 0.004\)), and accounted for 18% variability in brain A\textbeta{} change, comparable to that noted for the core CSF biomarkers CSF t-tau (standardized \(\beta\) = 0.31, \(R^2 = 0.19, P = .003\)) and p-tau (standardized \(\beta\) = 0.30, \(R^2 = 0.18, P = .004\)).

3.4 Association with likelihood or risk of amyloidopathy

Significantly higher levels of CSF FABP3 (mean [SD]) were noted in the individuals who were A\textbeta{}+ (n = 49, 3.27 [1.19] ng/ml) compared to A\textbeta{}– (n = 93; 2.58 [1.05] ng/ml, \(P = .001\)). Logistic regression analyses were used to assess the effect of elevated levels of FABP3 (as a categorical variable) on the likelihood or risk of amyloidopathy or A\textbeta{} positivity, OR, 95% CI (Table 3). Participants were classified as FABP3+ and FABP3– based on median measure as a cutoff (2.75 ng/ml), as well as the value corresponding to the best sensitivity and specificity (A\textbeta{}+ vs A\textbeta{}–) as a cutoff (2.85 ng/ml), determined using the ROC curve analysis. For median as a cutoff, FABP3 positivity (higher levels of CSF FABP3) was associated with the likelihood or risk of amyloidopathy (model 1; OR 2.28, 95% CI 1.12–4.65, \(P = .023\)). Associations were also assessed with inclusion of age, sex, and APOE 4 presence (model 2; OR 2.29, 95% CI 1.03–5.11, \(P = .042\)), as well as with only APOE 4 presence (model 3; OR 2.58, 95% CI 1.20–5.51, \(P = .015\)). The interaction of FABP3+ and APOE 4 presence was associated with a higher likelihood or risk of amyloidopathy (model 4; OR 3.15, 95% CI 1.12–8.89, \(P = .030\)).
For ROC cutoff (Aβ4+ vs Aβ−), FABP3 positivity (higher levels of CSF FABP3) was also associated with a likelihood or risk of amyloidopathy (model 1; OR 2.62, 95% CI 1.28–5.33, P = .008). Again, associations were also assessed with inclusion of age, sex, and APOE ε4 presence (model 2; OR 2.86, 95% CI 1.28–6.42, P = .011), as well as with only APOE ε4 presence (model 3; OR 3.11, 95% CI 1.44–6.73, P = .004). The interaction of FABP3+ and APOE ε4 presence was associated with a higher likelihood or risk of amyloidopathy (model 4; OR 3.26, 95% CI 1.09–9.79, P = .035).

4 | DISCUSSION

The inextricable association between lipid dyshomeostasis and AD neuropathology has been extensively studied and validated.28 FABP3 is a biomarker of neuronal membrane disruption, associated with lipid dyshomeostasis,32 whose potential for diagnosis of AD has been verified by several studies.13,16,29,30 Given the lack of evidence concerning the utility of CSF FABP3 for early diagnosis of AD, and given its association with AD risk factors, we tested the utility of CSF FABP3 to identify the likelihood of amyloidopathy. A positive association of elevated CSF FABP3 levels with brain Aβ load at baseline and change in Aβ load, found in this study, support the utility of this biomarker in identifying individuals who are likely in the preclinical phase of the AD continuum. Furthermore, we noted elevated levels of CSF FABP3 in individuals with Aβ pathology (Aβ+,), highlighting the potential of this biomarker for identifying AD-associated central pathophysiological changes in cognitively healthy individuals. Vidal-Pinerio et al. indicated that CSF levels of FABP3 predict brain atrophy among older cognitively healthy individuals, independent of biomarkers of amyloidopathy and tauopathy.31 Hoglund et al. noted higher levels of CSF FABP3 in cognitively healthy individuals at risk of developing AD—those who were CSF Aβ42. (CSF Aβ42 below the threshold).32 Desikan et al. indicated that elevation in CSF FABP3 levels reflect on Aβ-associated neurodegeneration in a cohort of demented and non-demented older individuals. They noted that elevated CSF FABP3 levels were associated with longitudinal brain atrophy only in individuals with Aβ pathology (low CSF Aβ42, Aβ+).29 Collectively, findings from these studies and the present data emphasize the likely association of elevated CSF FABP3 levels with Aβ pathology, which can be leveraged for making an early diagnosis of AD. Although our study focused on CSF FABP3, it would be worthwhile for future studies to evaluate their hypothesis by measuring blood FABP3 levels in cognitively healthy participants. Previously our team has reported elevated levels of FABP3 in plasma samples of AIBL participants (healthy controls vs AD and MCI).33 Moreover, given the association of blood FABP3 levels with AD risk factors—cardiovascular diseases18,19 and TBI17,34—blood levels of FABP3 could help to build sensitive biomarker-based risk-prediction models along with additional biomarkers associated with other risk factors or neuropathological changes. Future studies are needed to build and test such models for the early diagnosis of dementia. Such diagnostic models will involve a feasible and non-invasive approach of sample collection, enabling a population wide screening in a routine clinical setting. Such biomarker-based risk-prediction models can ascertain an “individual specific” magnitude of risk associated with developing AD/cognitive impairment and could improve diagnostic sensitivity.

Our results indicate that baseline levels of CSF FABP3 predict change in brain Aβ load, corroborating the utility of this biomarker as an indicator of future change in brain amyloidopathy. Therefore, higher levels of CSF FABP3, along with other biomarkers, will accurately predict risk of AD development in the vulnerable population (middle-aged and older individuals). Furthermore, our analyses indicate that higher levels of CSF FABP3 associate with a higher likelihood or odds of preclinical AD (defined by Aβ positivity). Results from a longitudinal study by Bjerke et al. have also revealed the predictive utility of CSF FABP3 for AD. They reported that elevated levels of FABP3 at baseline predicted the development of AD (OR 1.38, P = 0.019) in older women over 8 years of follow-up.35 Nonetheless, although in our study age and sex were found to influence CSF FABP3 levels, these covariates had minimal or negligible influence in mediating the effect of FABP3 on likelihood of preclinical AD as seen from the OR obtained upon controlling for all covariates. Th presence of the ε4 allele of APOE was found to have a positive effect on brain Aβ load—congruent with findings from other studies36,37—but had no influence in modulating CSF FABP3 levels. This indicates that elevation in CSF FABP3 (evidence of neurodegeneration/comorbid risk factors) and presence of APOE ε4 (evidence of genetic risk) independently influence brain Aβ amyloidopathy. FABP3 positivity (higher levels) and APOE ε4 presence together accounted for a higher likelihood of preclinical AD. Apparently, diagnostic models involving a combination of modifiable risk factors (accounted by biomarkers such as FABP3) and non-modifiable risk (accounted by genetic variants associated with the disease) could give an absolute prediction of likelihood of AD, help screen cases of preclinical AD with high accuracy, and predict cognitive decline among cognitively healthy individuals. Idland et al. noted that a combination of biomarkers including CSF FABP3 can help in improving prediction of cognitive decline among cognitively healthy individuals.38

The observed results in the current study, such as the estimated likelihood of amyloidopathy among cognitively healthy participants (high CSF FABP3 group vs low CSF FABP3) could have been overestimated and influenced by the small number of cognitively healthy participants. Future studies with larger sample numbers should be conducted to test the association.

In conclusion, findings from our study support that CSF FABP3 is a biomarker of early neurodegenerative changes. It can likely form an important component of sensitive risk-prediction models meant for early diagnosis of AD with Aβ asymptomatic amyloidosis, as well as predict change in brain Aβ load.

ACKNOWLEDGEMENTS

The authors thank the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) Study Group (www.aibl.csiro.au) and Edith Cowan University (ECU) and Deakin University. The AIBL is a collaboration between the Commonwealth Scientific and Industrial Research Organisation (CSIRO), ECU, The Florey Institute of Neuroscience and Mental Health (FINMH), National Ageing Research Institute (NARI),
and Austin Health. It involves support from CogState Ltd., Hollywood Private Hospital, and Sir Charles Gairdner Hospital. The initial core funding of the study was facilitated by CSIRO. The study receives funding from the National Health and Medical Research Council (NHMRC), the Dementia Collaborative Research Centres program (DCRC2), the Cooperative Research Centre (CRC) for Mental Health, the Australian Alzheimer’s Research Foundation, and Operational Infrastructure Support from the Government of Victoria. The authors also thank the participants for their assistance and commitment. KD also thanks ECU HDR (Higher degree by research) Scholarship. Veer Gupta is supported by NHMRC Boosting Dementia Research Leadership Fellowship #RM34909. KB is supported by the Swedish Research Council (#2017-00915), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), and the Alzheimer’s Association 2021 Zenith Award (ZEN-21-848495). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-0252), the European Research Council (#681712 and #101053962), Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer’s Association (#ADSF-21-831581-C, #ADSF-21-831381-C, and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), and the UK Dementia Research Institute at UCL (UKDRI-1003). SC receives an NHMRC Practitioner Fellowship (#APP1105784).

CONFLICT OF INTEREST
K.B. has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, BioArctic, 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. H.Z. has served as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectrocin, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). V.V. is and has been a consultant or paid speaker at sponsored conference sessions for Eli Lilly, Life Molecular Imaging, ACE Barcelona, GE Healthcare, IXICO, Hospicom, Abbvie, Lundbeck, Shanghai Green Valley Pharmaceutical Co Ltd, and Hoffmann La Roche. S.C. is currently a contracted adviser to Biogen. Other authors have no conflict of interests to disclose. Author disclosures are available in the Supporting Information.

REFERENCES
1. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of 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):280-292.
2. Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer’s disease: the IWG-2 criteria. Lancet Neurol. 2014;13(6):614-629.
3. Dubois B, Hampel H, Feldman HH, et al. Preclinical Alzheimer’s disease: definition, natural history, and diagnostic criteria. Alzheimers Dement. 2016;12(3):292-323.
4. Ashton NJ, Nevada-Holgado AJ, Barber IS, et al. A plasma protein classifier for predicting amyloid burden for preclinical Alzheimer’s disease. Sci Adv. 2019;5(2):eaau7220.
5. Golzan SM, Goooze K, Georgevsky D, et al. Retinal vascular and structural changes are associated with amyloid burden in the elderly: ophthalmic biomarkers of preclinical Alzheimer’s disease. Alzheimers Res Ther. 2017;9(1):13.
6. Harrington KD, Dang C, Lim Y, et al. The effect of preclinical Alzheimer’s disease on age-related changes in intelligence in cognitively normal older adults. Intelligence. 2018;70:22-29.
7. Insel PS, Weiner M, Mackin RS, et al. Determining clinically meaningful decline in preclinical Alzheimer disease. Neurology. 2019;93(4):e322.
8. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer’s disease. Alzheimers Dement. 2018;14(4):535-562.
9. Jack CR Jr, Bennett DA, Blennow K, et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016;87(5):539-547.
10. Dhiman K, Blennow K, Zetterberg H, Martins RN, Gupta VB. Cerebrospinal fluid biomarkers for understanding multiple aspects of Alzheimer’s disease pathogenesis. Cell Mol Life Sci. 2019;76(10):1833-1863.
11. Dhiman K, Villemagne VL, Fowler C, et al. Cerebrospinal fluid neurofilament light predicts risk of dementia onset in cognitively healthy individuals and rate of cognitive decline in mild cognitive impairment: a prospective longitudinal study. Biomedicines. 2022;10(5)1045
12. Sepe FN, Chiasserini D, Parnetti L. Role of FABP3 as biomarker in Alzheimer’s disease and synucleinopathies. Future Neurology. 2018;13(4):199-207.
13. Chiasserini D, Biscetti L, Eusebi P, et al. Differential role of CSF fatty acid binding protein 3, α-synuclein, and Alzheimer’s disease core biomarkers in Lewy body disorders and Alzheimer’s dementia. Alzheimers Res Ther. 2017;9(1):52.
14. Brosseron F, Kleemann K, Kolbe CC, et al. Interrelations of Alzheimer’s disease candidate biomarkers neurogranin, fatty acid-binding protein 3 and ferritin to neurodegeneration and neuroinflammation. J Neurochem. 2021;157(6):2210-2224.
15. Guo LH, Alexopoulos P, Perneeczky R. Heart-type fatty acid binding protein and vascular endothelial growth factor: cerebrospinal fluid biomarker candidates for Alzheimer’s disease. Eur Arch Psychiatry Clin Neurosci. 2013;263(7):553-560. https://doi.org/10.1007/s00406-013-0405-4
16. Olsson B, Hertz J, Olsson M, et al. Cerebrospinal fluid levels of heart fatty acid binding protein are elevated prodromally in Alzheimer’s disease and vascular dementia. J Alzheimers Dis. 2013;34(3):673-679. https://doi.org/10.3233/JAD-121384
17. Lagersstedt L, Azumendi L, Tenvouso O, et al. Interleukin 10 and heart fatty acid-binding protein as early outcome predictors in patients with traumatic brain injury. Front Neurol. 2020;11:376
18. Otaki Y, Watanabe T, Takahashi H, et al. Association of heart-type fatty acid-binding protein with cardiovascular risk factors and all-cause mortality in the general population: the Takahata study. PloS one. 2014;9(5):e94834.
19. Ho SK, Wu YW, Tseng WK, et al. The prognostic significance of heart-type fatty acid binding protein in patients with stable coronary heart disease. Sci Rep. 2018;8(1):14410.
20. Ellis KA, Bush AI, Darby D, et al. The Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging: methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer’s disease. Int Psychogeriatr. 2009;21(4):672-687. https://doi.org/10.1017/s1041610209009405
21. Clark CM, Schneider JA, Bedell BJ, et al. Use of florbetapir-PET for imaging β-amyloid pathology. JAMA. 2011;305(3):275-283.
22. Vandenbergh R, Van Laere K, Ivanoiu A, et al. 18F-flutemetamol amyloid imaging in Alzheimer disease and mild cognitive impairment: A phase 2 trial. Ann Neural. 2010;68(3):319-329.
23. Rowe CC, Ellis KA, Rimajova M, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Neurobiol Aging. 2010;31(8):1275-1283.
24. Bourgeat P, Villemagne VL, Dore V, et al. Comparison of MR-less PiB SUVR quantification methods. Neurobiol Aging. 2015;36 Suppl 1:S159-S166.
25. Villemagne VL, Doré V, Yates PA, et al. En attendant centiloid. 2014:2(12):723-729.
26. Jack CR Jr, Wiste HJ, Weigand SD, et al. Amyloid-first and neurodegeneration-first profiles characterize incident amyloid PET positivity. Neurology. 2013;81(20):1732-1740.
27. Vanderstichele H, Bibl M, Engelborghs S, et al. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer’s disease diagnosis: a consensus paper from the Alzheimer’s Biomarkers Standardization Initiative. Alzheimer Dement. 2012;8(1):65-73.
28. Chew H, Solomon VA, Fonteh AN. Involvement of lipids in Alzheimer’s disease pathology and potential therapies. Front Physiol. 2020;11:598.
29. Desikan RS, Thompson WK, Holland D, et al. Heart fatty acid binding protein and Aβ-associated Alzheimer’s neurodegeneration. Mol Neurodegener. 2013;8(3):39.
30. Chiasserini D, Parnetti L, Andreasson U, et al. CSF levels of heart fatty acid binding protein are altered during early phases of Alzheimer’s disease. J Alzhiemers Dis. 2010;22(4):1281-1288.
31. Vidal-Piñeiro D, Sørensen Ø, Blendow K, et al. Relationship between cerebrospinal fluid neurodegeneration biomarkers and temporal brain atrophy in cognitively healthy older adults. Neurobiol Aging. 2022;116:80-91.
32. Höglund K, Kern S, Zettergren A, et al. Preclinical amyloid pathology biomarker positivity: effects on tau pathology and neurodegeneration. Transl Psychiatry. 2017;7(1):e995-e995.
33. Gupta VB, Hone E, Pedrini S, et al. Altered levels of blood proteins in Alzheimer’s disease longitudinal study: results from Australian imaging biomarkers lifestyle study of ageing cohort. Alzheimer’s Dement (Amst). 2017;8:60-72.
34. Lagerstedt L, Egea-Guerrero JJ, Bustamante A, et al. H-FABP: a new biomarker to differentiate between CT-positive and CT-negative patients with mild traumatic brain injury. PLoS One. 2017;12(4):e0175572.
35. Bjerke M, Kern S, Blendow K, et al. Cerebrospinal fluid fatty acid-binding protein 3 is related to dementia development in a population-based sample of older adult women followed for 8 Years. J Alzheimer’s Dis. 2016;49(3):733-741.
36. Mishra S, Blazey TM, Holtzman DM, et al. Longitudinal brain imaging in preclinical Alzheimer disease: impact of APOE ε4 genotype. Brain. 2018;141(6):1828-1839.
37. Duara R, Loewenstein DA, Lizarraga G, et al. Effect of age, ethnicity, sex, cognitive status and APOE genotype on amyloid load and the threshold for amyloid positivity. Neuroimage Clin. 2019;22:101800.
38. Idland AV, Sala-Llonch R, Watne LO, et al. Biomarker profiling beyond amyloid and tau: cerebrospinal fluid markers, hippocampal atrophy, and memory change in cognitively unimpaired older adults. Neurobiol Aging. 2020;93:1-15.

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Dhiman K, Villemagne VL, Fowler C, et al. Cerebrospinal fluid levels of fatty acid-binding protein 3 are associated with likelihood of amyloidopathy in cognitively healthy individuals. Alzheimer’s Dement. 2022;14:e12377. https://doi.org/10.1002/dad2.12377