

## Original Investigation

# Predicting Reduction of Cerebrospinal Fluid $\beta$ -Amyloid 42 in Cognitively Healthy Controls

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**IMPORTANCE** Alzheimer disease has a long preclinical stage characterized by  $\beta$ -amyloid ( $A\beta$ ) accumulation without symptoms. Several trials focus on this stage and use biomarkers to include  $A\beta$ -positive participants, but an even earlier prevention of  $A\beta$  accumulation may be an effective treatment strategy.

**OBJECTIVE** To determine whether people who appear to be  $A\beta$  negative but are at high risk for  $A\beta$  positivity within the near future can be identified.

**DESIGN, SETTING, AND PARTICIPANTS** Longitudinal biomarker cohort study involving 35 cognitively healthy individuals who underwent cerebrospinal fluid (CSF) sampling for up to 3 years during the study (October 24, 2005, to September 1, 2014). All participants had normal CSF  $A\beta$ 42 levels at baseline.

**MAIN OUTCOMES AND MEASURES** Predictors of future  $A\beta$  positivity (levels of CSF  $A\beta$ 42 declining below a previously validated cutoff level of 192 ng/L) tested by random forest models. Tested predictors included levels of protein in the CSF, hippocampal volume, genetics, demographics, and cognitive scores.

**RESULTS** The CSF  $A\beta$ 42 levels declined in 11 participants, and the CSF became  $A\beta$  positive. The baseline CSF  $A\beta$ 42 level was a strong predictor of future positivity (accuracy, 79% [95% CI, 70%-87%]). Ten of 11 decliners had baseline CSF  $A\beta$ 42 levels in the lower tertile of the reference range (<225 ng/L), and 22 of 24 nondecliners had baseline CSF  $A\beta$ 42 levels in the upper 2 tertiles ( $\geq$ 225 ng/L). A high CSF P-tau level was associated with decline (accuracy, 68%; 95% CI, 55%-81%).

**CONCLUSIONS AND RELEVANCE** Baseline CSF  $A\beta$ 42 levels in the lower part of the reference range are strongly associated with future  $A\beta$  positivity. This finding can be used in trials on very early prevention of Alzheimer disease to identify people at high risk for  $A\beta$  accumulation as defined by low CSF  $A\beta$ 42 levels.

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**A**lzheimer disease (AD) is believed to have a long preclinical stage before apparent cognitive impairment.<sup>1</sup> This stage may last for decades and is characterized by the presence of asymptomatic brain pathology, primarily  $\beta$ -amyloid ( $A\beta$ ) accumulation. The accumulation can be detected by biomarkers in the form of decreased cerebrospinal fluid (CSF) levels of  $A\beta$ 42<sup>2</sup> and increased uptake of  $A\beta$  ligands visualized by positron emission tomography (PET).<sup>3</sup> The  $A\beta$  biomarkers are important for drug development since they enable trials to be conducted in people with  $A\beta$  accumulation in the symptomatic (tertiary prevention) or asymptomatic (secondary prevention) stage of the disease. In the absence of successful anti- $A\beta$  trials, it remains unknown at what stage anti- $A\beta$

treatment must be administered to be effective. One possibility is that the most effective approach would be to inhibit the initial accumulation of  $A\beta$ . If so, drug trials would need to be carried out in asymptomatic individuals who appear to lack amyloid accumulation but who are at high risk of developing it within a few years. Here we used longitudinal CSF  $A\beta$ 42 to model the development of  $A\beta$  accumulation in cognitively healthy people. The indication of decline was defined as CSF  $A\beta$ 42 crossing the threshold of 192 ng/L, which corresponds to  $A\beta$  positivity in imaging<sup>4</sup> and autopsy<sup>5,6</sup> studies. It is often assumed that  $A\beta$  biomarker changes occur before other biomarker changes in AD,<sup>1</sup> but some studies<sup>7,8</sup> have suggested that other brain changes (especially tau related) may precede  $A\beta$

accumulation. We therefore tested the hypothesis that baseline CSF A $\beta$ 42, biomarkers of neurodegeneration, demographic factors, and cognitive scores could be used to identify people at risk for A $\beta$  accumulation as determined by low CSF A $\beta$ 42 levels, within 3 years.

## Methods

### Study Design

Data used in the preparation of this article were obtained from the Alzheimer Disease Neuroimaging Initiative (ADNI) database (<http://adni.loni.usc.edu>). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the US Food and Drug Administration, private pharmaceutical companies, and non-profit organizations as a \$60 million, 5-year, public-private partnership. The principal investigator of this initiative is Michael W. Weiner, MD, Veterans Affairs Medical Center and University of California, San Francisco. The ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and participants have been recruited from more than 50 sites across the United States and Canada. The initial goal of the ADNI was to recruit 800 individuals, but the ADNI has been followed by ADNI-Grand Opportunity (ADNI-GO) and ADNI-2. To date, these 3 protocols have enrolled more than 1500 adults aged 55 to 90 years to participate in the research, consisting of cognitively normal older individuals, people with early or late mild cognitive impairment, and people with early AD. The follow-up duration of each group is specified in the protocols for ADNI-1, ADNI-GO, and ADNI-2. Individuals originally recruited for ADNI-1 and ADNI-GO had the option to be monitored in ADNI-2.<sup>9</sup> The present study was conducted from October 24, 2005, to September 1, 2014. Institutional review board approval was received at all involved sites. Written informed consent was obtained from all participants.

### Cohort

Our study population consisted of cognitively healthy individuals serving as controls enrolled in ADNI-1 or ADNI-2. Inclusion and exclusion criteria have been described in detail.<sup>9</sup> Briefly, all control participants included in ADNI were between the ages of 55 and 90 years, had completed at least 6 years of education, were fluent in Spanish or English, were free of any significant neurologic disease, had a Mini-Mental State Examination<sup>10</sup> score of 24 or higher, and had a Clinical Dementia Rating scale<sup>11</sup> score of 0. For the present study, we only included those with available longitudinal data on CSF A $\beta$ 42 levels and baseline CSF A $\beta$ 42 levels indicating a lack of A $\beta$  accumulation at baseline as determined by a validated cutoff level (192 ng/L).

### CSF Biomarkers

All participants underwent CSF sampling at baseline and at least once (yearly or every second year) in a follow-up visit during 3 years (or shorter in 5 individuals who became A $\beta$  positive during follow-up). Measurement of levels of A $\beta$ 42, T-tau, and P-

tau was performed using the multiplex xMAP Luminex platform (Luminex Corp) with the INNO-BIA AlzBio3 kit (Innogenetics) as described previously.<sup>5,12</sup> Longitudinal samples for each participant were analyzed on the same plate in the same analytical run.<sup>13</sup> Within-run coefficients of variation were 6.5% or less. Parts of the longitudinal data have been published.<sup>13</sup>

### Cognition

The Alzheimer Disease Assessment Scale-Cognitive Subscale, version 11 (ADAS-cog11),<sup>14</sup> logical memory delayed recall, Rey Auditory Verbal Learning Test (AVLT) delayed recall,<sup>15</sup> and Trail Making Test, part B (Trail B)<sup>16</sup> were performed at baseline. For analysis of longitudinal cognitive scores, we used up to 8 years of follow-up data.

### Structural Magnetic Resonance Imaging

Brain scans using T1-weighted structural magnetic resonance imaging (MRI) were acquired at baseline with 1.5-T (ADNI-1) or 3-T (ADNI-2) MRI scanners using a sagittal volumetric magnetization-prepared rapid gradient echo nonaccelerated sequence.<sup>17</sup> Quantification was done in an automated pipeline using FreeSurfer (version 4.3 for ADNI-1 and version 5.1 for ADNI-2, <http://surfer.nmr.mgh.harvard.edu/fswiki>).<sup>18,19</sup> Individuals whose quality control test results were below standard were excluded. Data on hippocampal volume (mean of right and left) were used. Hippocampal volume was adjusted for total intracranial volume. Because the acquisition and processing of images differed between ADNI-1 and ADNI-2, the data from these study groups were analyzed separately. Individuals with an adjusted hippocampal volume below the 25th percentile within each study group were labeled as having small hippocampal volume. The choice of the 25th percentile was a trade-off between achieving adequate group sizes and a level of hippocampal volume that may be associated with early-stage brain injury.

### PET Imaging

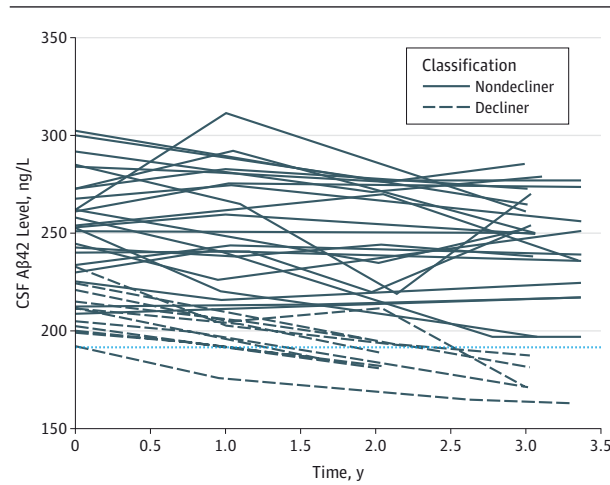
We included data on ADNI-1 participants who had undergone carbon 11(<sup>11</sup>C)-labeled Pittsburgh compound B PET scanning and ADNI-2 participants who had undergone florbetapir F 18 PET scanning to test for the presence of brain A $\beta$  accumulation as estimated by PET (using previously established cutoffs for <sup>11</sup>C-Pittsburgh compound B, 1.47 standardized uptake volume ratio [SUVR],<sup>20</sup> and <sup>18</sup>F-florbetapir, 1.11 SUVR<sup>21-23</sup>).

### Statistical Analysis

Baseline biomarkers (levels of CSF A $\beta$ 42, CSF T-tau, and CSF P-tau and small hippocampal volume), demographic factors (age, sex, and educational level), genetics (presence of APOE  $\epsilon$ 4 and APOE  $\epsilon$ 2 alleles), and cognitive scores (ADAS-cog11, AVLT, Trail B, and logical memory delayed recall) were compared between participants with vs without CSF A $\beta$ 42 (termed *decliners* and *nondecliners*, respectively) using Mann-Whitney tests and  $\chi^2$  tests. Longitudinal cognition was examined by linear mixed-effects models adjusted for age, sex, and educational level.

The binary classification of CSF A $\beta$ 42 decline vs nondecline was the dependent variable in random forest analyses

**Figure 1. Longitudinal Cerebrospinal Fluid (CSF)  $\beta$ -Amyloid 42 (A $\beta$ 42) Levels**



Individual lines indicate whether participants were classified as individuals whose CSF A $\beta$ 42 level declined (decliners) or did not decline (nondecliners) within 3 years. Some individuals lacked data at the 3-year time point. For these (indicated by lines extending beyond the 3-year point), we used data at 4 or 5 years to determine their likely status at 3 years. Dashed horizontal line indicates the cutoff level (192 ng/L).

with predictors of baseline biomarkers, demographic factors, genetics, and cognitive scores. The predictors were tested individually and in combination (individually significant predictors were combined). Random forest models use an ensemble of decision trees for classification (here we used 3000 trees in each model based on an inspection of stabilization of the random forest out-of-bag [OOB] error, which is the percentage of accurate predictions<sup>24</sup>). The prediction for an individual is determined by a simple majority vote among the trees. We generated votes for each participant in a 5-fold cross-validation procedure (the analysis was iterated 5 times, each time including 80% of the data in the training set and predicting the remaining 20%). The final diagnostic accuracy for each predictor was determined by the random forest OOB value. We calculated the mean and the 95% CI of the OOB value from the 5 cross-validation iterations. If this 95% CI did not include 0.5, we concluded that the predictor was significantly associated with decline. For continuous predictors, results were visualized by receiver operating characteristic curves (using the cross-validated votes and the observed outcome). All statistical evaluations were done using R, version 3.1.1 (The R Foundation for Statistical Computing).

## Results

### Classification of CSF A $\beta$ 42 Decliners

We classified participants as CSF A $\beta$ 42 decliners if the observed CSF A $\beta$ 42 level became lower than 192 ng/L within 3 years. Those whose A $\beta$ 42 level remained negative at 3 years were classified as nondecliners. Ten individuals lacked data at exactly 3 years but had data available at 4 years, 5 years, or both. These participants were classified as *nondecliners* if the

4- to 5-year follow-up sample was negative ( $n = 9$ ) and as *decliners* if both the 2- and 4-year follow-up samples were positive ( $n = 1$ ). No participants had the potentially ambiguous combination of a negative 2-year sample and a positive 4-year sample; one person in the original data set had samples at baseline, 1 year (negative), and 6 years (positive) and was excluded from all analyses because his status at 3 years was ambiguous. **Figure 1** shows the observed CSF A $\beta$ 42 data, and the **Table** presents the demographics and baseline biomarker data and cognitive scores. The only significant difference between decliners and nondecliners was the lower baseline CSF A $\beta$ 42 levels in the decliners. There were no significant differences between the CSF A $\beta$ 42 groups in longitudinal trajectories of cognitive scores ( $P > .05$ ).

### Predicting CSF A $\beta$ 42 Decline

We performed random forest analyses with decliners vs nondecliners as the dependent variable and demographic factors, baseline biomarker data, and cognitive scores as predictors. The results are presented as OOB in the **Table** and as ROC curves for continuous predictors in **Figure 2** (receiver operating characteristic curves are not meaningful for dichotomous predictors). Baseline CSF A $\beta$ 42 level was the strongest individual predictor of future decline (OOB accuracy, 79%; 95% CI, 70%-87%). The high diagnostic accuracy for baseline CSF A $\beta$ 42 was in agreement with the distribution of baseline CSF A $\beta$ 42 level in decliners and nondecliners (**Figure 1** and the **Table**). The individual decision trees for CSF A $\beta$ 42 favored splits at a level corresponding to the lower tertile of baseline CSF A $\beta$ 42 (225 ng/L). Among the 12 participants with baseline CSF A $\beta$ 42 levels less than 225 ng/L, 10 were decliners (positive predictive value, 83%). Among the 23 participants with baseline CSF A $\beta$ 42 levels of 225 ng/L or more, 22 were nondecliners (negative predictive value, 96%).

Baseline CSF P-tau levels also significantly predicted future decline (OOB accuracy, 68% [95% CI, 55%-81%]). The individual decision trees for CSF P-tau favored splits at CSF P-tau 25 ng/L, which was close to the upper tertile of baseline CSF P-tau (26 ng/L) and also close to a previously suggested cutoff for CSF P-tau to identify AD (23 ng/L, when analyzed at the same laboratory and with the same assays as in the ADNI study<sup>25</sup>). Among the 10 participants with baseline CSF P-tau levels higher than 25 ng/L, 6 were decliners (positive predictive value, 60%). Among the 25 individuals with baseline CSF P-tau levels less than 25 ng/L, 20 were nondecliners (negative predictive value, 80%).

Sex, *APOE*  $\epsilon$ 2, *APOE*  $\epsilon$ 4, and small hippocampal volume also appeared to be significant individual predictors (OOB accuracy, 64%-69%) (**Table**). However, closer inspection of these random forest models revealed that the models classified all but one participant as nondecliners, and their OOB accuracy therefore corresponds to the percentage of nondecliners in the examined population.

We performed additional random forest analyses testing a combination of CSF A $\beta$ 42 and P-tau, which had an OOB accuracy rate of 84% (95% CI, 72%-97%). We also tried combinations of CSF A $\beta$ 42, P-tau, and other predictors. The best accuracy was seen for a combination of CSF A $\beta$ 42, P-tau, and the

Table. Characteristics of the Study Population

Characteristic	Mean (SD)			P Value <sup>a</sup>	Out-of-Bag Accuracy (95% CI) <sup>b</sup>
	Total (N = 35)	CSF A $\beta$ 42 Decliners (n = 11)	CSF A $\beta$ 42 Nondecliners (n = 24)		
Age, y	76.1 (6.0)	76.0 (5.8)	76.1 (6.2)	.96	0.45 (0.32-0.58)
Education, y	15.7 (3.0)	16.0 (2.6)	15.5 (3.3)	.86	0.56 (0.45-0.68)
Sex, No. (%)					
Male	17 (49)	4 (36)	13 (54)	.54	0.69 (0.65-0.72)
Female	18 (51)	7 (64)	11 (46)		
APOE $\epsilon$ 2, No. (%)					
Positive	7 (20)	1 (9)	6 (25)	.52	0.64 (0.57-0.71)
Negative	28 (80)	10 (90)	18 (75)		
APOE $\epsilon$ 4, No. (%)					
Positive	4 (11)	2 (18)	2 (8)	.78	0.69 (0.65-0.72)
Negative	31 (89)	9 (82)	22 (92)		
Cognition					
ADAS-cog	5.0 (2.3)	5.6 (2.8)	4.7 (2.0)	.35	0.59 (0.44-0.73)
Trail B	79.6 (24.7)	87.3 (31.8)	76.1 (20.6)	.34	0.54 (0.40-0.68)
Logical memory delayed recall	12.8 (3.8)	12.8 (3.3)	12.8 (4.0)	.89	0.58 (0.36-0.80)
AVLT delayed recall	8.5 (3.0)	7.6 (2.9)	9.0 (2.9)	.11	0.56 (0.39-0.73)
Baseline biomarkers					
CSF A $\beta$ 42, ng/L	242 (31)	211 (12)	257 (25)	<.001	0.79 (0.70-0.87)
CSF T-tau, ng/L	63 (21)	71 (25)	59 (18)	.15	0.45 (0.35-0.55)
CSF P-tau, ng/L	23 (7.9)	25 (7.9)	21 (7.8)	.16	0.68 (0.55-0.81)
Small hippocampus, No. (%) <sup>c</sup>					
True	10 (29)	3 (27)	7 (29)	>.99	0.68 (0.65-0.71)
False	24 (69)	8 (73)	16 (67)		

Abbreviations: A $\beta$ ,  $\beta$ -amyloid; ADAS-cog, Alzheimer Disease Assessment Scale–Cognitive Subscale; AVLT, Auditory Verbal Learning Test; CSF, cerebrospinal fluid; Trail B, Trail Making Test, part B.

<sup>a</sup> P values are for comparisons between emerging A $\beta$  accumulation (decliners) and the nondecliners (Mann-Whitney tests and  $\chi^2$  tests).

<sup>b</sup> Out-of-bag accuracies (ranging from 0 to 1, where 1 is perfect accuracy) are from random forest analyses. Seven APOE  $\epsilon$ 2-positive participants had 1 APOE  $\epsilon$ 2 and 1 APOE  $\epsilon$ 3 allele. Three APOE  $\epsilon$ 4-positive participants had 1 APOE  $\epsilon$ 4 and 1 APOE  $\epsilon$ 3 allele and one individual had 1 APOE  $\epsilon$ 2 allele. For sex, APOE, and small hippocampus, the out-of-bag accuracy appears to be significant, but closer inspection of these models showed that they classified all cases (or all but 1 case) as nondecliners, and these accuracies therefore represent the frequency of nondecliners in the population.

<sup>c</sup> Information was missing on 1 participant.

presence of APOE  $\epsilon$ 2, which had an OOB accuracy rate of 87% (95% CI, 74%-100%).

### Comparison with A $\beta$ PET

Eight participants had A $\beta$  PET data at the start of the study (5 at baseline and 3 at the 1-year follow-up). One of these individuals was PET positive (<sup>18</sup>F-florbetapir SUVR, 1.21; CSF A $\beta$ 42, 201 ng/L at baseline and 182 ng/L at follow-up). Follow-up PET data during the study period were available for 6 CSF A $\beta$ 42 decliners and 2 nondecliners; none became PET positive, and there was no change in the one participant with PET-positive findings.

## Discussion

The main finding of this study was that it is possible to predict declining levels of CSF A $\beta$ 42 in cognitively healthy individuals monitored for 3 years. *Declining CSF A $\beta$ 42* was defined as the CSF A $\beta$ 42 level falling below the a priori-defined threshold of 192 ng/L. Baseline CSF A $\beta$ 42 level was a strong predictor of declining CSF A $\beta$ 42. Individuals with CSF A $\beta$ 42 levels in the lower tertile of the reference range were very likely to become A $\beta$  positive during follow-up. The 192 ng/L cutoff was initially derived from a sample of patients with autopsy-confirmed AD dementia vs nonautopsy-confirmed controls.<sup>5</sup> This threshold, and corresponding thresholds used for A $\beta$  PET

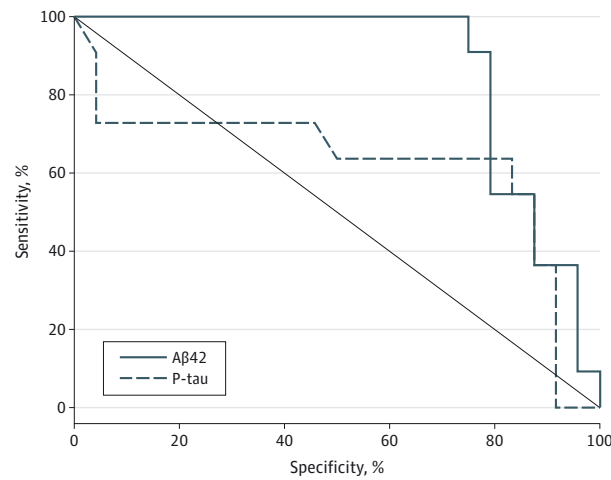
imaging, may be too stringent to detect very early pathology. The findings of our study suggest that a more liberal (higher) cutoff level could be explored in cognitively healthy controls.

Previous studies<sup>26</sup> have described a large variability in CSF A $\beta$ 42 levels among healthy controls, even for individuals who have CSF A $\beta$ 42 levels above the normal cutoff value. Theoretically, this variability could be the result of a combination of factors, including differences in production and clearance of the A $\beta$  peptide. Thus, CSF A $\beta$ 42 levels in the low normal range could be due to a physiologically low A $\beta$  production rate, which may not be associated with further decline. However, our finding contradicts this hypothesis and suggests that a low normal CSF A $\beta$ 42 level is rarely a benign phenomenon since it is strongly associated with future CSF A $\beta$ 42 decline. The finding agrees with previous results using A $\beta$  PET, in which cognitively healthy people who became A $\beta$  positive during follow-up had a higher baseline A $\beta$  signal than people who remained A $\beta$  negative,<sup>27</sup> which implies an association between A $\beta$  PET levels that are within the reference range and neurodegeneration.<sup>28</sup>

The recent failures of anti-amyloid drug trials, such as the phase 3 trials of bapineuzumab<sup>29</sup> and solanezumab,<sup>30</sup> have been blamed partly on the fact that some of the participants did not have A $\beta$  accumulation.<sup>31</sup> In addition, the disease may be too advanced in patients with dementia for optimal intervention via an anti-amyloid mechanism since downstream effects of amyloid accumulation, especially tau pathology, may become self-propagating at some point.<sup>32</sup> This theory is



**Figure 2. Baseline Predictors of Longitudinal Cerebrospinal Fluid (CSF)  $\beta$ -Amyloid 42 (A $\beta$ 42) Decline**



The receiver operating characteristic curves were generated by cross-validated predictions from random forest models using different variables (biomarkers, demographics, and cognitive scores) to predict longitudinal CSF A $\beta$ 42 level decline vs nondecline. The A $\beta$ 42 and P-tau accuracies were significantly different from 50% (as measured by random forest out-of-bag error).

supported by some of the autopsy data from the AN1792 trial,<sup>33</sup> in which amyloid immunization may have removed amyloid plaques without affecting the course of the disease. Several new trials therefore use A $\beta$  biomarkers to verify that participants have amyloid accumulation, both for tertiary and secondary prevention, and/or focus on patients with less severe clinical disease.<sup>34,35</sup> These results suggest that an even earlier prevention—before A $\beta$  biomarkers have reached the cutoff normally associated with widespread pathology—could also be an attractive approach since there are several problems associated with treatment when pathology is established. For example, successful prevention in people with established amyloid accumulation may be hampered by a large variability in the length of time that individuals have been exposed to the toxic effects of amyloid. Some people may have become amyloid positive very recently and others may have had amyloid depositions for years, putting them at higher risk for imminent spread of tau pathology and cognitive impairment. However, the benefits of primary prevention must be weighed against possible risks, costs, and the availability of early screening and intervention.

There has been discussion about whether A $\beta$  or neurodegeneration appears first during the course of AD.<sup>7,8,27</sup> Although the present study was not specifically aimed at answering this question, our results suggest that signs of A $\beta$  accumulation and signs of tau-related neurodegeneration may appear early in the disease process since a high baseline CSF P-tau level was also a significant predictor of future A $\beta$  level decline. Complicating analyses of preclinical A $\beta$  and neurodegeneration, brain A $\beta$  may have very early effects on neurodegeneration, even before A $\beta$  biomarkers become clearly positive.<sup>36</sup> The ADNI investigators<sup>37</sup> recently found that hippocampal atrophy rates started to accelerate at CSF A $\beta$ 42 levels of approxi-

mately 220 ng/L,<sup>37</sup> which was about the same level of CSF A $\beta$ 42 that was associated with a high risk of declining CSF A $\beta$ 42 in the present study. Together, these findings suggest that the emergence of A $\beta$  accumulation and neurodegeneration is coupled even early (from a clinical perspective) in the disease process. Of course, these conclusions are based on currently available biomarker tools. For example, CSF A $\beta$ 42 may lack sensitivity for the most toxic forms of brain amyloid.

This study has several limitations. The main limitation is the small sample size, especially of CSF A $\beta$ 42 decliners, which restricts our power to identify significant predictors. The results therefore need to be confirmed in larger studies. Furthermore, the participants had a mean age of 76 years, and primary prevention of A $\beta$  accumulation should be explored in younger individuals given the long duration of preclinical amyloid accumulation.<sup>38,39</sup> The study group included few APOE  $\epsilon$ 4 carriers since most elderly APOE  $\epsilon$ 4 carriers are CSF A $\beta$ 42 positive at baseline. Thus, our results may not be generalizable to APOE  $\epsilon$ 4 carriers. Another possible limitation is the inclusion of individuals who were close to the cutoff level at baseline, which may lead to a bias by those crossing the threshold on the basis of random variation. However, the decliners decreased by a mean (SD) of 14.2% (5.9%) in CSF A $\beta$ 42 levels from baseline to their last measurement, which clearly exceeded the intra-assay variability for CSF A $\beta$ 42 (coefficient of variation,  $\leq 6.5^{12,13}$ ). In contrast, although some participants who were classified as nondecliners had negative slopes, the mean (SD) change in the nondecliner group was within the intra-assay variability ( $-5.3\%$  [9.5%]). This argues against random variability having a major effect on the classification of the participants. The fact that individuals who were classified as decliners had both lower baseline values and steeper decline of CSF A $\beta$ 42 levels than did those who were classified as nondecliners supports the idea that CSF A $\beta$ 42 levels may have a sigmoidal trajectory, as previously suggested.<sup>40</sup> Our definition of decliners did not include people whose CSF A $\beta$ 42 level decreased without reaching the cutoff value (192 ng/L) within 3 years. Although some of those individuals may be moving toward A $\beta$  accumulation, excluding them from the decliner group reflects possible procedures in future trials aimed at preventing A $\beta$  accumulation. We only analyzed hippocampal volume as a predictor of future CSF A $\beta$ 42 level decline, but future studies may also explore other structures, including the precuneus, entorhinal, lateral temporal, and lateral parietal cortices. Likewise, several other cognitive tests could be explored. Recently, a composite score for secondary prevention AD trials was presented (Alzheimer's Disease Cooperative Study-Preclinical Alzheimer Cognitive Composite).<sup>41</sup> However, the ADNI procedures allow only an approximation of this score, and the effects of baseline A $\beta$  level on the score were very mild in ADNI controls<sup>41</sup>; therefore, we did not include the score in the present study. Finally, we could not confirm A $\beta$  accumulation with an independent technology. Few participants in this group underwent PET A $\beta$  imaging, and there was no evidence of PET A $\beta$  positivity in the CSF A $\beta$ 42 decliners. This is difficult to interpret given the small number of people with PET imaging results available, but previous reports<sup>42,43</sup> have suggested that CSF A $\beta$ 42 levels may be reduced before PET A $\beta$  posi-

tivity is observed. Therefore, a longer follow-up period would be necessary to determine PET evidence of  $A\beta$  accumulation in this group.

## Conclusions

Our study shows that it is possible to identify individuals at risk for  $A\beta$  accumulation. Such people are primarily charac-

terized by CSF  $A\beta_{42}$  levels slightly above the cutoff level of 192 ng/L. This finding is in agreement with a previously proposed model of sigmoidal  $A\beta$  biomarker trajectories,<sup>40</sup> as well as with findings using  $A\beta$  PET,<sup>27</sup> and adds to a previous determination that CSF proteins may be used to predict longitudinal reduction of CSF  $A\beta_{42}$ .<sup>44</sup> Individuals with CSF  $A\beta_{42}$  levels in the low reference range may be optimal candidates for early intervention trials aimed at thwarting further  $A\beta$  accumulation.

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**Author Contributions:** Dr Mattsson had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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*Study supervision:* Jagust, Weiner.

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Disease conference; serves as an associate editor of *Alzheimer's & Dementia*; has received honoraria from Danone Trading BV, Pfizer, and Tohoku University; has received research support from Avid, Merck, the US Department of Defense, and the Veterans Administration; and has stock options in Elan and Synarc. No other conflicts were reported.

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**Group Information:** A complete listing of the ADNI investigators can be found in the eAppendix in the Supplement.

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