

# High Soluble Amyloid- $\beta_{42}$ Predicts Normal Cognition in Amyloid-Positive Individuals with Alzheimer's Disease-Causing Mutations

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**Abstract.**

**Background:** In amyloid-positive individuals at risk for Alzheimer's disease (AD), high soluble 42-amino acid amyloid- $\beta$  (A $\beta_{42}$ ) levels are associated with normal cognition. It is unknown if this relationship applies longitudinally in a genetic cohort.

**Objective:** To test the hypothesis that high A $\beta_{42}$  preserves normal cognition in amyloid-positive individuals with Alzheimer's disease (AD)-causing mutations (*APP*, *PSEN1*, or *PSEN2*) to a greater extent than lower levels of brain amyloid, cerebrospinal fluid (CSF) phosphorylated tau (p-tau), or total tau (t-tau).

**Methods:** Cognitive progression was defined as any increase in Clinical Dementia Rating (CDR = 0, normal cognition; 0.5, very mild dementia; 1, mild dementia) over 3 years. Amyloid-positivity was defined as a standard uptake value ratio (SUVR)  $\geq 1.42$  by Pittsburgh compound-B positron emission tomography (PiB-PET). We used modified Poisson regression models to estimate relative risk (RR), adjusted for age at onset, sex, education, *APOE4* status, and duration of follow-up. The results were confirmed with multiple sensitivity analyses, including Cox regression.

**Results:** Of 232 mutation carriers, 108 were PiB-PET-positive at baseline, with 43 (39.8%) meeting criteria for progression after  $3.3 \pm 2.0$  years. Soluble A $\beta_{42}$  levels were higher among CDR non-progressors than CDR progressors. Higher A $\beta_{42}$  predicted a lower risk of progression (adjusted RR, 0.36; 95% confidence interval [CI], 0.19–0.67;  $p = 0.002$ ) better than lower SUVR (RR, 0.81; 95% CI, 0.68–0.96;  $p = 0.018$ ). CSF A $\beta_{42}$  levels predicting lower risk of progression increased with higher SUVR levels.

**Conclusion:** High CSF A $\beta_{42}$  levels predict normal cognition in amyloid-positive individuals with AD-causing genetic mutations.

**Keywords:** Alzheimer's disease, amyloid- $\beta$ , atrophy, cognition, FDG-PET

## INTRODUCTION

Key support for the toxic amyloid hypothesis comes from the observation that mutations in any of three genes (*APP*, *PSEN1*, and *PSEN2*) lead to Alzheimer's disease (AD) with complete penetrance [1]. The genetic evidence causally implicates the fibrillogenic 42-amino acid amyloid- $\beta$  peptide (A $\beta_{42}$ ). However, the disease pathogenesis may arise from either of two ends of the protein aggregation process: the increase in insoluble amyloid plaques or the depletion of the soluble A $\beta_{42}$  peptide, which has important functions. While insoluble amyloid plaques can be present in normal individuals, low soluble levels of A $\beta_{42}$  are an invariable feature of AD [2–4].

The hypothesis of A $\beta$  toxicity has traditionally been supported by the notion that AD-causing mutation carriers must have *high* levels of soluble A $\beta_{42}$  relative to non-mutation populations. In fact, mutation carriers have *lower* A $\beta_{42}$  levels compared to non-mutation populations [3]. The reduction in soluble A $\beta_{42}$  levels among mutation carriers begins as many as 25 years before the onset of cognitive symptoms [4]. Therefore, the toxicity in the process of accelerated protein aggregation among mutation carriers may conceivably be due to the depletion in soluble A $\beta_{42}$  to a greater extent than the corresponding increase in amyloid [5]. This alternative hypothesis offers an explanation for the failures in

translating amyloid reduction into cognitive improvement [6], even among mutation carriers [7], and for the paradoxes posed by the large proportion of amyloid-positive individuals without dementia and even of centenarians without history of cognitive abnormalities, half of whom have autopsy-confirmed AD pathology [8–10].

We recently observed that among amyloid positron emission tomography (PET)-positive individuals, higher levels of soluble A $\beta_{42}$  were associated with normal cognition and brain volumes in all tertiles of brain amyloidosis, with an effect size greater than that of increases in brain amyloid burden [11]. We here tested the hypothesis that in amyloid PET-positive individuals with AD-causing *APP*, *PSEN1*, or *PSEN2* mutations, higher CSF A $\beta_{42}$  levels reduce the risk of cognitive progression to a greater extent than lower levels of brain amyloidosis and lower levels of CSF phosphorylated tau (p-tau) and total tau (t-tau).

## METHODS

### Overview

We conducted a retrospective longitudinal study among mutation carriers participating in the Dominantly Inherited Alzheimer Network (DIAN) cohort study. The DIAN study longitudinally evaluates families with dominantly inherited AD and non-carrier relatives of the probands. Details about the DIAN

study design can be found in previous publications [1, 12]. We included all mutation carriers with at least two follow-up visits including baseline, without applying any other inclusion/exclusion criteria. All analyses were conducted in two cohorts: 1) amyloid PET-positive cohort, the most critical as these are individuals considered at greater risk for developing dementia; and 2) overall cohort, which included both amyloid PET-positive and amyloid PET-negative subjects.

### *Clinical assessment*

Each assessment comprised a detailed medical history, neurological, and neuropsychological examination. Cognitive function was primarily quantified using the Clinical Dementia Rating scale (CDR = 0, indicates normal cognition; 0.5, very mild dementia; 1, mild dementia; 2, moderate dementia; and 3, severe dementia) [13]. Our primary endpoint was CDR progression, defined as any increase in CDR over the follow-up period. Secondary endpoints were progression to CDR  $\geq 0.5$  confirmed in two consecutive visits as per DIAN guidelines to minimize misclassification, progression to CDR  $\geq 1$ , Mini-Mental State Examination (MMSE) score  $\leq 24$  at last visit (higher means better cognition) [14], and a CDR sum of boxes (CDR-SB) score  $\geq 4.5$  at last visit (lower means better cognition) [15]. CDR-SB = 4.5 corresponds to mild dementia [15].

### *CSF biomarkers*

We evaluated CSF A $\beta_{42}$ , p-tau, and t-tau levels (INNO-BIA AlzBio3 from Fujirebio, Malvern, PA). In order to limit the variability due to measurement error, we considered only data from the same assay, as used in previous analyses [4]. While CSF A $\beta_{40}$  was not available from this particular assay, precluding us from entering it into our analysis, it did not limit the testing of our hypothesis since A $\beta_{40}$  exhibits lower fibrillogenicity and lesser depletion than A $\beta_{42}$  [16], and is therefore less relevant to the process of protein aggregation than A $\beta_{42}$ .

### *Neuroimaging*

Pittsburgh compound B PET (PiB-PET) was used to quantify the burden of insoluble brain amyloid plaques. To reduce partial volume effects, data were processed using a regional spread function (RSF), shown to enhance sensitivity [17]. We defined sub-

jects as amyloid PiB-PET-positive if their standard uptake value ratio (SUVR) was  $\geq 1.42$  [18], validated by using +5% higher SUVR threshold ( $\geq 1.49$ ) as a sensitivity analysis. At the last visit, we also used RSF-processed fluorodeoxyglucose (FDG)-PET to quantify the metabolism of the precuneus (average of both hemispheres) and brain magnetic resonance imaging (MRI) to quantify hippocampal volume (average of both hemispheres) adjusted for intracranial volume, using the DIAN protocol equation.

### *Aims and sample size calculation*

Our primary aim was to test the hypothesis that higher CSF A $\beta_{42}$  at baseline reduces the risk of CDR progression to a greater extent than lower SUVR, lower p-tau, and lower t-tau levels among PiB-PET-positive gene carriers. Secondly, we tested whether compared with lower baseline SUVR, p-tau, and t-tau levels, higher baseline CSF A $\beta_{42}$  predict lower risk of progression to CDR  $\geq 0.5$  in at least two consecutive visits, progression to CDR  $\geq 1$  at any visit, MMSE  $\leq 24$  at last visit, CDR-SB  $\geq 4.5$  at last visit, and higher FDG-PET metabolism and hippocampi volume at last visit. While these aims were focused on the PiB-PET-positive cohort, we conducted similar analyses on the full cohort, which included PiB-PET-negative participants.

### *Sample size calculation*

In a previous cross-sectional study of PiB-PET-positive individuals, we found that higher CSF A $\beta_{42}$  levels were associated with greater odds of normal cognition than AD (adjusted odds ratio [OR], 6.26;  $p < 0.001$ ) or mild cognitive impairment (OR, 1.42;  $p = 0.006$ ) [11]. Using these results, a sample size of 105 PiB-PET-positive mutation carriers was calculated to yield >80% power to detect a significant association (OR  $> 1.85$ ) between soluble A $\beta_{42}$  levels (assuming continuous normal distribution) and the primary (CDR progression) and secondary endpoints (progression to CDR  $\geq 0.5$ , progression to CDR  $\geq 1$ , MMSE  $\leq 24$ , CDR-SB  $\geq 4.5$ ) using multiple logistic regression with a two-sided Wald test after adjusting for other independent variables with a coefficient of determination ( $R^2$ ) of 15% and baseline probability of outcome of 50%. Such an estimated sample was also sufficient to explore weak effect-size associations (regression coefficient, 0.30–0.60) between CSF A $\beta_{42}$  levels and other secondary outcomes (FDG-SUVR and hippocampal volume) with more than 80% power at a 5% alpha using mul-

multiple linear regression after adjusting for relevant covariates. Sample size estimation and simulations were carried out using PASS 2021 (Power Analysis and Sample Size Software [2021]. NCSS, LLC. Kaysville, UT, USA, [ncss.com/software/pass](http://ncss.com/software/pass)).

### Statistical analysis

Continuous standardized CSF and SUVR levels were considered primary predictors in the analyses for a direct comparison across effects of CSF and SUVR levels. For the primary outcome of CDR progression, we used a modified Poisson model regression [19] to determine the associations of baseline CSF A $\beta_{42}$ , CSF t-tau and p-tau, and SUVR levels with CDR progression after adjusting for age at onset (estimated mean age of symptom onset), sex, education, *APOE4*, and duration of follow-up. The age at onset was estimated for participants by the site investigators. We also validated our results by considering time to first CDR progression as an outcome using Cox proportional hazards regression analysis. A receiver operating characteristics curve analysis was used to determine the CSF A $\beta_{42}$  cut-off along with its area under the curve, sensitivity, and specificity in predicting CDR progression. The binary secondary outcomes (progression to CDR  $\geq 0.5$ , progression to CDR  $\geq 1$ , MMSE  $\leq 24$ , CDR-SB  $\geq 4.5$ ) were analyzed using relative risk (RR) regression [19], whereas the quantitative secondary outcomes (FDG-PET and normalized hippocampal volume) using multiple linear regression. Finally, in addition to adjusting for CDR-SB and MMSE at baseline, all the regression models were adjusted for the above-described covariates and validated by removing t-tau or p-tau to minimize the impact of multicollinearity. Huber Sandwich estimator was used to compute standard errors of the estimates in all the Cox, relative risk, and linear regression models. As a sensitivity analysis, we conducted the same analysis after removing subjects within 5% of the threshold (SUVR  $\geq 1.49$ ). The results of the Cox regression analysis were summarized with hazard ratio (HR) and 95% confidence interval (CI), those of relative risk regression with RR and 95% CI, and those of linear regression with regression coefficient (RC) and 95% CI. The effect sizes and *p*-values were qualitatively compared among CSF and SUVR levels. The fully-detailed statistical analysis, statistical analysis codes, and sample size estimation are provided in the Supplementary Material.

### Ethics approval

The study protocol for DIAN was approved by the local ethical committees of all participating institutions. The DIAN study was conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from each participant.

## RESULTS

Of 534 subjects participating in the DIAN study (mean age,  $38 \pm 11.1$  years), 232 mutation carriers met eligibility criteria (mean age,  $38.3 \pm 11$  years). Among them, 191 had available PiB-PET data at baseline (mean SUVR,  $1.9 \pm 1.0$ ), of whom 108 were PiB-PET-positive (mean SUVR,  $2.5 \pm 1.0$ ) (Table 1). These subjects were followed for a mean of  $3.3 \pm 2.0$  years (range = 1, 9). Because of missing data on some CSF and SUVR levels, multivariable analyses were based on 93 PiB-PET-positive samples, 85 PiB-PET-positive samples (SUVR  $\geq 1.49$ ), and 162 overall samples which include PiB-PET-positive and negative samples (Supplementary Figure 1). No differences in the baseline characteristics except CDR at baseline were observed between cohorts with missing versus without missing data (Supplementary Table 1).

### Primary endpoint

#### Amyloid PiB-PET-positive cohort

A total of 43 (39.8%) subjects met criteria for CDR progression. In adjusted analyses, the risk of progression was reduced to a greater extent by higher CSF A $\beta_{42}$  (RR, 0.36; 95% CI, 0.19–0.67;  $p=0.002$ ) than lower SUVR (RR, 0.81; 95% CI, 0.68–0.96;  $p=0.018$ ) but not than lower t-tau or lower p-tau (Fig. 1, top). These results were unchanged after excluding 5% of the SUVR threshold. In addition, higher CSF A $\beta_{42}$  levels were associated with a reduced hazard of time to first CDR progression (HR, 0.37; 95% CI, 0.18–0.77;  $p=0.008$ ) to a greater extent than lower SUVR (HR, 0.79; 95% CI, 0.62–1.02;  $p=0.075$ ) and lower p-tau (HR, 0.63; 95% CI, 0.40–0.98;  $p=0.041$ ). These results were confirmed in the stratified Cox model (Supplementary Table 2). CSF A $\beta_{42}$  levels predicted progression to a greater extent than SUVR, CSF t-tau, or CSF p-tau levels in all analyses excluding p-tau or t-tau from the models to address multicollinearity, after additionally adjusting for baseline CDR and age, or after missing imputations (Supplementary Tables 3 and 4).

Table 1  
Characteristics of study cohort

	PiB-PET-positive cohort (n = 108)	Overall cohort (n = 232)
Age (y)	41.0 (10.4)	38.3 (11.0)
Sex (female)	55 (50.9%)	130 (56.0%)
Education (y)	13.8 (3.0)	14.3 (3.0)
APOE4 carriers	40 (37.0%)	72 (31.0%)
CSF A $\beta_{42}$ (pg/ml)	264.6 (107.9) <sup>a</sup>	350.6 (186.8) <sup>a</sup>
SUVr (amyloid-PiB-PET)	2.5 (1.0) <sup>b</sup>	1.9 (1.0) <sup>b</sup>
t-tau (pg/ml)	144.5 (89.4) <sup>c1</sup>	111.2 (81.6) <sup>c1</sup>
p-tau (pg/ml)	81.7 (38.1) <sup>c2</sup>	61.9 (37.6) <sup>c2</sup>
CDR at baseline		
0	55 (50.9%)	147 (63.4%)
0.5	33 (30.6%)	60 (25.9%)
1	17 (15.7%)	20 (8.6%)
2	2 (1.9%)	3 (1.3%)
3	1 (0.9%)	2 (0.9%)
CDR progression		
No	65 (60.2%)	165 (71.1%)
Yes	43 (39.8%)	67 (28.9%)
CDR (two consecutive visits)		
<0.5	53 (49.1%)	150 (64.7%)
≥0.5	55 (50.9%)	82 (35.3%)
CDR (any visit)		
<1	74 (68.5%)	176 (75.9%)
≥1	34 (31.5%)	56 (24.1%)
CDR-SB at baseline	1.8 (2.8) <sup>d</sup>	1.2 (2.5) <sup>d</sup>
CDR-SB at last visit	3.7 (5.0) <sup>d</sup>	2.7 (4.7) <sup>d</sup>
≥4.5	74 (68.5%)	178 (76.7%)
<4.5	34 (31.5%)	54 (23.3%)
MMSE at baseline	26.4 (4.9) <sup>e1</sup>	27.1 (4.4) <sup>e1</sup>
MMSE at last visit	23.4 (7.7) <sup>e2</sup>	25.1 (7.2) <sup>e2</sup>
≤24	69 (63.9%)	174 (75.0%)
>24	39 (36.1%)	58 (25.0%)
FDG-PET at baseline (SUVr)	1.8 (0.3) <sup>f1</sup>	1.8 (0.2) <sup>f1</sup>
FDG-PET last visit (SUVr)	1.7 (0.3) <sup>f2</sup>	1.8 (0.3) <sup>f2</sup>
Average hippocampi baseline (mm <sup>3</sup> )	4022.4 (708.7) <sup>g1</sup>	4174.6 (626.0) <sup>g1</sup>
Average hippocampi last visit	3795.2 (767.6) <sup>g2</sup>	4039.4 (741.3) <sup>g2</sup>
Normalized hippocampi baseline (mm <sup>3</sup> )	4022.3 (709.6) <sup>g1</sup>	4174.3 (627.0) <sup>g1</sup>
Normalized hippocampi last visit (mm <sup>3</sup> )	3795.2 (767.6) <sup>g2</sup>	4039.4 (741.3) <sup>g2</sup>

n, number of subjects; APOE4, Apolipoprotein ε4; CDR, clinical dementia rating; CDR-SB, CDR sum of boxes; CSF, cerebrospinal fluid; A $\beta_{42}$ , 42-amino acid amyloid-beta peptide; t-tau, total tau; p-tau, phosphorylated-tau; PiB-PET, Pittsburgh compound B positron emission tomography; SUVr, standardized uptake value ratio; MMSE, Mini-Mental State Examination; FDG, fluorodeoxyglucose; pg, picogram; ml, milliliter; mm, millimeter. Data are expressed in mean  $\pm$  standard deviation (SD) or frequency (%). <sup>a</sup>CSF A $\beta_{42}$  data were available for 191 subjects and 93 PiB-PET-positive. <sup>b</sup>SUVr data were available for 191 subjects and 108 PiB-PET-positive. <sup>c1</sup>CSF t-tau data were available for 194 subjects and 95 PiB-PET-positive. <sup>c2</sup>CSF p-tau data were available for 268 subjects and 122 PiB-PET-positive. <sup>d</sup>CDR-SB data were available for 232 subjects and 108 PiB-PET-positive. <sup>e1</sup>MMSE baseline data were available for 231 subjects and 107 PiB-PET-positive. <sup>e2</sup>MMSE last follow-up were available for 232 subjects and 108 PET-positive. <sup>f1</sup>FDG-PET data at baseline were available for 196 subjects and 102 PET-positive. <sup>f2</sup>FDG-PET at last visit were available for 172 subjects and 92 PET-positive. <sup>g1</sup>MRI baseline data were available for 215 subjects and 108 PET-positive. <sup>g2</sup>MRI last visit were available for 200 subjects and 98 PET-positive.

### Overall cohort

A total of 67 (28.9%) subjects met criteria for CDR progression in the overall cohort. Higher CSF A $\beta_{42}$  levels predicted a reduced risk of progression (RR, 0.47; 95% CI, 0.29–0.76;  $p=0.002$ ) to a greater extent than lower SUVr (RR, 0.80; 95% CI, 0.67–0.97;  $p=0.021$ ), but not than lower t-tau or p-tau (Fig. 1, bottom). The results were unchanged after

additionally adjusting for baseline CDR and age in all analyses with or without p-tau or t-tau or after missing imputations (Supplementary Tables 3 and 4). Moreover, higher CSF A $\beta_{42}$  levels were associated a reduced hazard of time to first CDR progression (HR, 0.51; 95% CI, 0.31–0.86;  $p=0.011$ ) than lower SUVr (HR, 0.79; 95% CI, 0.62–1.01;  $p=0.062$ ) and lower p-tau (HR, 0.62; 95% CI, 0.40–0.97;  $p=0.037$ ).

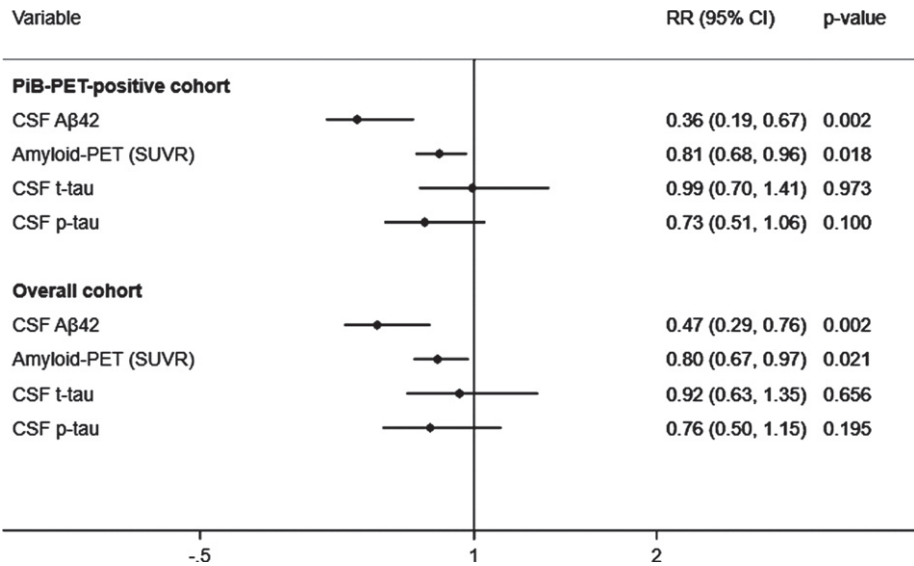


Fig. 1. Adjusted prediction of CDR progression with baseline CSF Aβ<sub>42</sub>, p-tau, t-tau, and amyloid (PiB)-PET SUVR levels. CSF, cerebrospinal fluid; PiB-PET, Pittsburgh compound B positron emission tomography; SUVR, standardized uptake value ratio; CDR, Clinical Dementia Rating; Aβ<sub>42</sub>, 42-amino acid amyloid-beta peptide; p-tau, phospho-Tau; t-tau, total-Tau; HR, hazard ratio; CI, confidence interval. RR reflects effect size for the association with a one standard deviation higher in CSF Aβ<sub>42</sub> levels and lower in SUVR, CSF t-tau and p-tau levels. Analyses were adjusted for age at onset, sex, education, APOE4, and duration of follow up. Overall cohort includes PiB-PET-positive and negative samples.

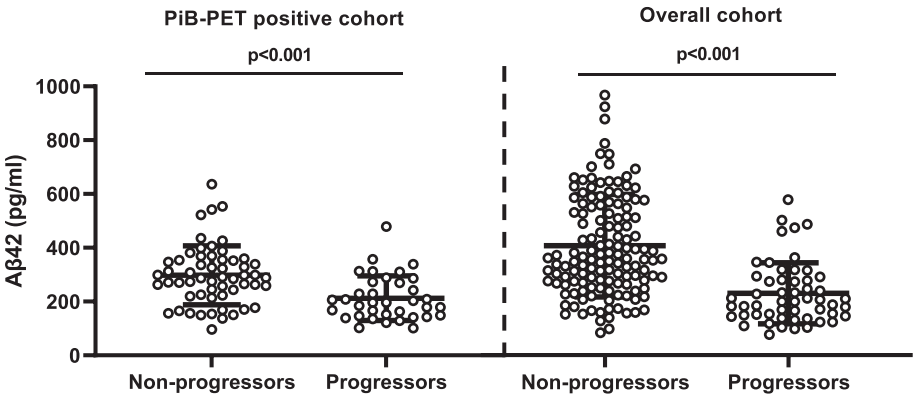


Fig. 2. Comparison of CSF Aβ<sub>42</sub> levels between non-progressors and progressors. PiB-PET-positive cohort: non-CDR progressors (297.73 ± 13.66) versus CDR progressors (218.73 ± 17.22); overall cohort: non-CDR progressors (380.83 ± 14.5) versus CDR progressors (313.35 ± 26.46). Error bar represents the standard error of mean. CSF, cerebrospinal fluid; PiB-PET, Pittsburgh compound B positron emission tomography; CDR, Clinical Dementia Rating; Aβ<sub>42</sub>, 42-amino acid amyloid-beta peptide. Overall cohort includes PiB-PET-positive and negative samples.

These results were confirmed in the stratified Cox model (Supplementary Table 2).

CSF Aβ<sub>42</sub> and CDR progression

CSF Aβ<sub>42</sub> levels were higher among CDR non-progressors than CDR progressors (Fig. 2). CSF Aβ<sub>42</sub> <270 pg/ml predicted progression (area under the curve, 80.5%; sensitivity, 72.2%; specificity,

74.5%) regardless of increasing SUVR levels. The progression-free survival was longer with CSF Aβ<sub>42</sub> ≥270 pg/ml compared to CSF Aβ<sub>42</sub> <270 pg/ml over the follow-up period in both PiB-PET-positive (*p* = 0.002) and overall cohorts (*p* < 0.001) (Supplementary Figure 2). In adjusted analysis, higher CSF Aβ<sub>42</sub> levels reduced the risk of progression even at very high PiB-PET SUVR levels. CSF Aβ<sub>42</sub> levels predicting lower risk of progression increased with higher SUVR levels (Fig. 3).

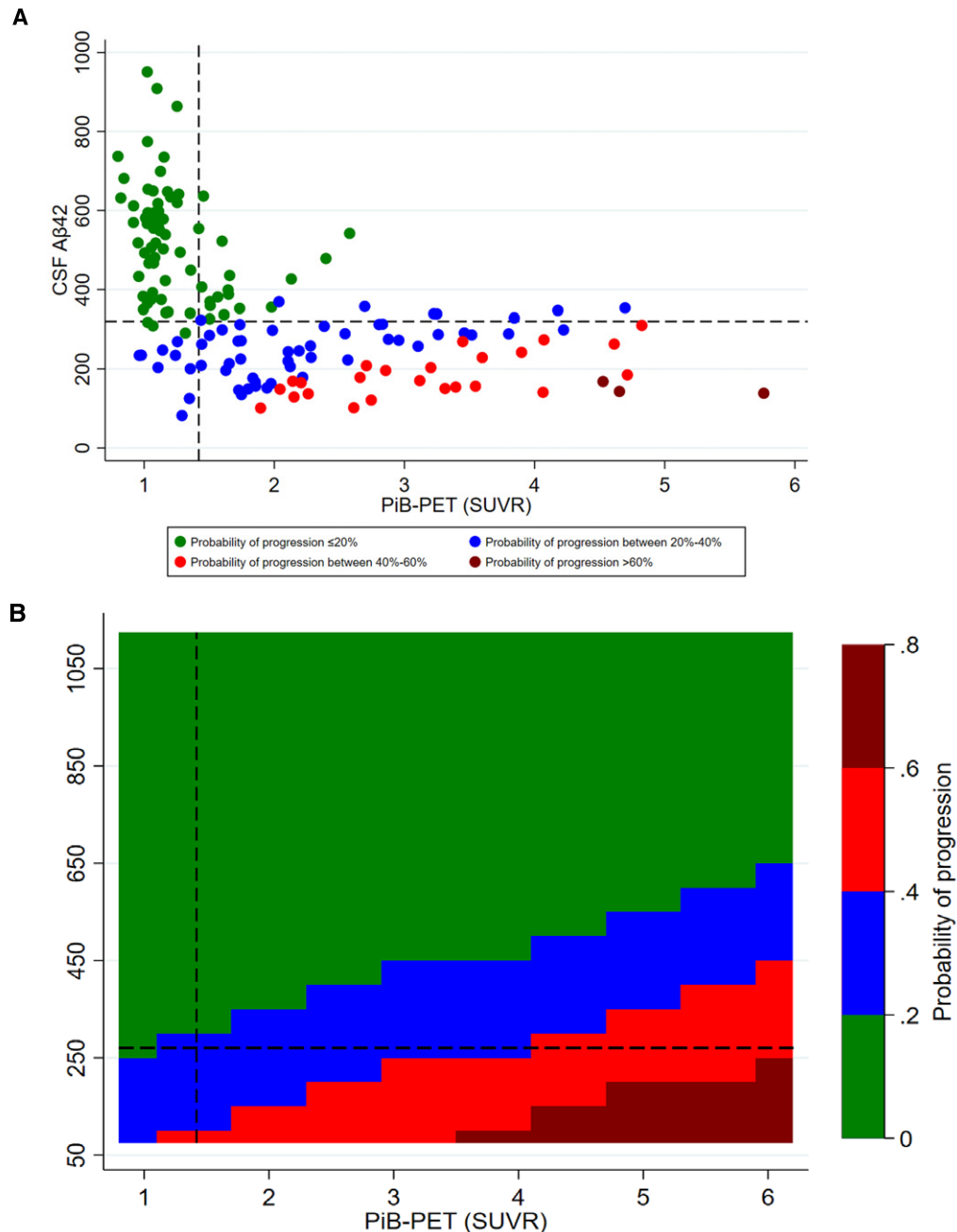


Fig. 3. Adjusted probability of CDR progression. A) scatter plot of CSF A $\beta_{42}$  and PiB-PET SUVR levels; B) contour plot of CSF A $\beta_{42}$  and PiB-PET SUVR levels. CSF, cerebrospinal fluid; A $\beta_{42}$ , 42-amino acid amyloid-beta peptide; PiB-PET, Pittsburgh compound B positron emission tomography; CDR, Clinical Dementia Rating; p-tau, phospho-Tau; t-tau, total-Tau. All models were adjusted for age at onset, sex, education, APOE4, p-tau, and t-tau levels. Overall cohort includes PiB-PET-positive and negative samples.

### Secondary endpoints

#### Amyloid PiB-PET-positive cohort

Higher baseline CSF A $\beta_{42}$  predicted a reduced risk of progression to CDR  $\geq 0.5$  (RR, 0.55; 95%

CI, 0.36–0.83;  $p=0.004$ ) and to CDR  $\geq 1$  (RR, 0.27; 95% CI, 0.13–0.59;  $p=0.001$ ), and was associated with CDR-SB  $\geq 4.5$  (RR, 0.36; 95% CI, 0.36–0.18–0.70;  $p=0.003$ ) and MMSE  $\leq 24$  (RR, 0.30; 95% CI, 0.17–0.54;  $p<0.001$ ) better than

Table 2  
Adjusted associations of baseline CSF and SUVR with secondary cognitive outcomes

	PiB-PET-positive cohort				PiB-PET-positive cohort (SUVR $\geq 1.49$ )				Overall cohort			
	RR*	95% CI		p	RR*	95% CI		p	RR*	95% CI		p
Progression to CDR $\geq 0.5$												
CSF A $\beta_{42}$	0.55	0.36	0.83	0.004	0.59	0.38	0.91	0.017	0.52	0.35	0.77	0.001
SUVR (PiB-PET)	0.75	0.63	0.90	0.002	0.78	0.65	0.93	0.006	0.70	0.59	0.84	<0.001
CSF t-tau	0.97	0.78	1.19	0.764	0.96	0.78	1.19	0.693	0.98	0.78	1.23	0.876
CSF p-tau	0.67	0.52	0.86	0.002	0.69	0.53	0.89	0.005	0.60	0.46	0.79	<0.001
Progression to CDR $\geq 1$												
CSF A $\beta_{42}$	0.27	0.13	0.59	0.001	0.29	0.13	0.62	0.002	0.26	0.13	0.51	<0.001
SUVR (PiB-PET)	0.72	0.55	0.94	0.016	0.74	0.56	0.97	0.028	0.66	0.51	0.87	0.003
CSF t-tau	0.95	0.74	1.23	0.724	0.94	0.74	1.22	0.681	0.96	0.74	1.25	0.751
CSF p-tau	0.57	0.36	0.89	0.015	0.59	0.37	0.92	0.020	0.50	0.32	0.78	0.002
CDR-SB $\geq 4.5$ at last visit <sup>#</sup>												
CSF A $\beta_{42}$	0.36	0.18	0.70	0.003	0.38	0.19	0.74	0.005	0.32	0.18	0.58	<0.001
SUVR (PiB-PET)	0.78	0.63	0.96	0.018	0.79	0.64	0.98	0.030	0.73	0.59	0.90	0.004
CSF t-tau	1.09	0.85	1.39	0.492	1.09	0.85	1.37	0.520	1.12	0.88	1.43	0.366
CSF p-tau	0.47	0.29	0.75	0.002	0.48	0.30	0.76	0.002	0.40	0.25	0.64	<0.001
MMSE $\leq 24$ at last visit <sup>#</sup>												
CSF A $\beta_{42}$	0.30	0.17	0.54	<0.001	0.32	0.18	0.59	<0.001	0.26	0.15	0.45	<0.001
SUVR (PiB-PET)	0.81	0.64	1.03	0.094	0.83	0.66	1.06	0.135	0.75	0.59	0.96	0.022
CSF t-tau	0.98	0.69	1.39	0.931	0.97	0.70	1.35	0.872	1.02	0.72	1.45	0.921
CSF p-tau	0.61	0.40	0.94	0.026	0.64	0.42	0.97	0.036	0.52	0.34	0.81	0.003

\*Relative risk (RR) is with a one standard deviation higher in CSF A $\beta_{42}$  levels and lower in CSF t-tau, CSF p-tau, and SUVR levels. CI, confidence interval; CDR, clinical dementia rating; CSF, cerebrospinal fluid; A $\beta_{42}$ , 42-amino acid amyloid-beta peptide; t-tau, total tau; p-tau, phospho-Tau; CDR-SB, CDR sum of boxes; PiB-PET, Pittsburgh compound B positron emission tomography; SUVR, standardized uptake value ratio; MMSE, Mini-Mental State Examination; FDG, fluorodeoxyglucose. All CSF and SUVR values are standardized. Analysis adjusted for mean mutation age of symptom onset, sex, education, APOE4 status, and duration of follow up. <sup>#</sup>Analyses were also adjusted for baseline CDR-SB or MMSE; Overall cohort includes PiB-PET-positive and negative samples.

lower SUVR, lower t-tau, or lower p-tau levels (Table 2). Higher CSF A $\beta_{42}$  levels were also associated with larger hippocampi volume and higher FDG-PET uptake at baseline to a greater extent than lower SUVR, lower p-tau or lower t-tau levels (data not shown). These results were unchanged in age-adjusted models with other covariates and after removing t-tau and p-tau from the models (Supplementary Tables 5 and 6). Higher baseline CSF A $\beta_{42}$  levels were associated with larger hippocampal volume (RC, 319.91; 95% CI, 73.97–565.86;  $p=0.012$ ) and higher FDG-PET uptake (RC, 0.14; 95% CI, 0.03–0.24;  $p=0.011$ ) to a greater extent than lower PiB-PET SUVR levels (Supplementary Table 7). The results were unchanged after excluding 5% of the SUVR threshold and in age-adjusted models with other covariates (Supplementary Table 7). The association between CSF A $\beta_{42}$  levels and all secondary endpoints remained significant after excluding t-tau or p-tau (Supplementary Table 8).

#### Overall cohort

Higher baseline CSF A $\beta_{42}$  predicted a reduced risk of progression to CDR  $\geq 0.5$  (RR, 0.52; 95% CI, 0.35, 0.77;  $p=0.001$ ) and to CDR  $\geq 1$  (RR, 0.26;

95% CI, 0.13, 0.51;  $p<0.001$ ), and was associated with CDR-SB  $\geq 4.5$  (RR, 0.32; 95% CI, 0.18, 0.58;  $p<0.001$ ) and MMSE  $\leq 24$  (RR, 0.26; 95% CI, 0.15, 0.45;  $p<0.001$ ) better than lower SUVR, lower t-tau, or lower p-tau levels (Table 2). Similar results were observed in age-adjusted models with other covariates (Supplementary Table 5) and models with and without p-tau or t-tau (Supplementary Table 6). Larger hippocampal volume was inversely associated with SUVR levels (RC, –204.70; 95% CI, –341.07, –68.33;  $p=0.004$ ) and p-tau levels (RC, –219.06; 95% CI, –405.74, –32.37;  $p=0.022$ ) but not with CSF A $\beta_{42}$  levels (Supplementary Table 7). Higher SUVR was associated with lower FDG-PET uptake (RC, –0.07; 95% CI, –0.13, –0.01;  $p=0.022$ ) but not with CSF A $\beta_{42}$  (Supplementary Tables 7 and 8).

## DISCUSSION

This longitudinal analysis of a genetic cohort showed that in amyloid PiB-PET-positive individuals with autosomal dominant AD-causing mutations **higher levels of CSF A $\beta_{42}$  were associated with reduced risk of progression to cognitive impairment,**



as well as larger hippocampal volume and higher brain metabolism in the precuneus. Conversely, cognitive deterioration was strongly predicted by lower levels of soluble A $\beta_{42}$  but not by increases in SUVR, t-tau, or p-tau. These data are in agreement with those of a cross-sectional analysis of a sporadic AD cohort (in participants of the Alzheimer's Disease Neuroimaging Inventory cohort study), in which higher CSF A $\beta_{42}$  levels were associated with normal cognition regardless of (and despite increasing) SUVR levels among PiB-PET-positive individuals [11], making a reduction in CSF A $\beta_{42}$  levels a better marker of progression than higher levels of CSF t-tau, p-tau, or amyloid PiB-PET SUVR.

A threshold of compensation can tentatively be drawn from these data, with further studies needed to validating it. We found that levels of CSF A $\beta_{42}$  above 270 pg/ml predicted a reduced risk of CDR progression across any levels of PiB-PET SUVR. The better prediction of disease conversion by the reduction of CSF A $\beta_{42}$  levels than by the increase in brain amyloidosis is in line with its observed decline as early as 25 years before the onset of cognitive impairment in genetic AD [4]. There is also support for a greater pathophysiologic importance of the loss of soluble A $\beta_{42}$  over the accrual of insoluble PiB-PET amyloid from knockout animal models of the A $\beta_{42}$  precursor, A $\beta$ PP, which yields neurodegeneration without brain amyloidosis [20], and in AD patients treated with BACE-1 inhibitors, which reduced CSF A $\beta_{42}$  levels and worsened cognitive symptoms regardless of changes in brain amyloid [21]. *In vitro* studies have shown that *PSEN* mutations reduce the levels of A $\beta_{42}$  and A $\beta_{40}$  [22], even if some mutations increase the A $\beta_{42/40}$  ratio [23, 24]. Clinically, however, mutation carriers, including Down syndrome patients with *APP* duplications, have lower CSF levels of A $\beta_{42}$  and A $\beta_{42/40}$  ratio at the symptomatic stage compared to cognitively unimpaired individuals [3]. Supporting the importance of the soluble protein fraction, individuals with dementia associated with the rare *APP* E693del (Osaka) mutation exhibit low CSF A $\beta_{42}$  despite no PiB-PET-amyloid positivity [25]. Importantly, the inverse correlation between the progressive loss of soluble A $\beta_{42}$  along with the increase in insoluble A $\beta$  is imperfect. In fact, we previously showed that soluble A $\beta_{42}$  levels can still be high even in individuals with the highest amyloid-SUVR PiB-PET tertile, a relationship associated with normal cognition [11].

Amyloids represent the end product of an irreversible phase transition process of proteins,

changing from a soluble to an insoluble state via a physicochemical process termed nucleation [26]. Nucleation is the rate-limiting step of the first stable amyloid cluster (nucleus), after which the phase transition proceeds spontaneously until all the available soluble substrate is consumed. While nucleation is not favorable under normal conditions, it becomes favorable at higher concentrations, such as those attained via gene duplication, or when the protein is rendered unstable due to structural mutations [27]. The net outcome is decreased availability of soluble A $\beta_{42}$ . This explains why their depletion is universal across all reported familial AD mutations, including *APP* duplications [3, 28]. Whereas an increase in amyloid plaque burden in the brain exhibits an inconsistent relationship with cognitive impairment, the depletion of soluble A $\beta_{42}$  is universally associated with dementia in all familial and sporadic forms of AD, further suggesting it is pathophysiologically more relevant [1, 3, 4, 29]. This is supported by an extensive body of literature demonstrating the role of physiological (picomolar) concentrations of A $\beta_{42}$  in memory and synaptic plasticity via  $\alpha$ -7 nicotinic acetylcholine receptor ( $\alpha$ -7-nAChR) signaling (Supplementary Table 9). Very recently, synthetic A $\beta_{42}$  monomers have been shown to improve impaired memory in conditional double knockout mice without plaque deposition and in the *APP/PS1/Tau* triple transgenic mice with A $\beta_{42}$  deposition, effects that were mediated by the  $\alpha$ -7-nAChR [30]. These results not only indicate the importance of the loss of A $\beta_{42}$  function as a pathogenic mechanism both in the presence and absence of plaques, but also suggest that A $\beta_{42}$  replacement has therapeutic potential.

Major strengths of this analysis are its longitudinal design and the relatively large number of subjects with disease-causing mutations. The findings are supported by the use of several complementary analytic methods on cognitive assessments and neuroimaging data and confirmed by sensitivity analyses. Major limitations include the inability to adjust for the estimated year to symptom onset (highly correlated with CSF and SUVR levels and did not serve as a confounder), and the lack of data on CSF A $\beta_{40}$  (and therefore of A $\beta_{42}/\text{A}\beta_{40}$  ratio; see below) and on oligomeric species. On the last point, subjects with normal cognition despite high PiB-PET plaque burden had high, not low CSF A $\beta_{42}$  [11], opposite to the direction predicted by the hypothesis of oligomeric toxicity. Oligomers are very transient and the majority of them dissociate back to monomers rather than

progress to aggregates [31]. Importantly, the reduction of soluble A $\beta_{42}$  levels during the disease course reduces the substrate for the oligomers, further questioning any sustainable toxicity from them.

A note related to the issue of ratio versus absolute values is worth making. Early studies argued that absolute levels of A $\beta_{42}$  decrease but the A $\beta_{42}$ /A $\beta_{40}$  ratio *increases* due to the lower decrease of A $\beta_{40}$  relative the more aggregation-prone A $\beta_{42}$  [32, 33]. However, recent studies have found that the A $\beta_{42}$ /A $\beta_{40}$  ratio *decreases* in AD [3, 34]. Pathophysiologically, amyloid aggregation is dependent on supersaturation [35–37], which is based on the absolute concentration of the peptide. Decreasing peptide concentration will decrease, not increase, the saturation and the related propensity to form any type of aggregates irrespective of its relative levels compared to other peptides [38]. Nearly 90% of AD-related mutations in the *PSEN1* gene lead to reduced production of both A $\beta_{42}$  and A $\beta_{40}$  [22], a classical genetic loss-of-function mechanism. Further, there is no correlation between the A $\beta_{42}$ /A $\beta_{40}$  ratio and the age of onset in mutation carriers [22].

**In conclusion, higher soluble A $\beta_{42}$  levels are associated with reduced risk of CDR progression, normal cognition, normal hippocampal volume, and normal precuneus metabolism to a greater extent than lower brain amyloid, lower p-tau, and lower t-tau levels in amyloid PiB-PET-positive individuals with autosomal dominant AD-causing genetic mutations. Brain toxicity in AD may be predominantly mediated by a reduction of the soluble protein pool, its functional fraction, rather than its accrual into amyloids.**

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## DATA SHARING

All data are available upon request from the Dominantly Inherited Alzheimer Network (DIAN) through the study website, <https://dian.wustl.edu/>.

## SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <https://dx.doi.org/10.3233/JAD-220808>.

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