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Featured Article

Hypothesis: cerebrospinal fluid protein markers suggest a pathway toward symptomatic resilience to AD pathology

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Abstract

Introduction: We sought biological pathways that explained discordance between Alzheimer's disease (AD) pathology and symptoms.

Methods: In 306 Alzheimer's Disease Neuroimaging Initiative (ADNI)-1 participants across the AD clinical spectrum, we investigated association between cognitive outcomes and 23 cerebrospinal fluid (CSF) analytes associated with abnormalities in the AD biomarkers amyloid $\beta_{1\text{-}42}$ and total-*tau*. In a 200-person "training" set, Least Absolute Shrinkage and Selection Operator regression estimated model weights for the 23 proteins, and for the AD biomarkers themselves, as predictors of ADAS-Cog_{11} scores. In the remaining 106 participants ("validation" set), fully adjusted regression models then tested the Least Absolute Shrinkage and Selection Operator–derived models and a related protein marker summary score as predictors of ADAS-Cog_{11}, ADNI diagnostic category, and longitudinal cognitive trajectory.

Results: AD biomarkers alone explained 26% of the variance in validation set cognitive scores. Surprisingly, the 23 AD-related proteins explained 31% of this variance. The biomarkers and protein markers appeared independent in this respect, jointly explaining 42% of test score variance. The composite protein marker score also predicted ADNI diagnosis and subsequent cognitive trajectory. Cognitive outcome prediction redounded principally to ten markers related to lipid or vascular functions or to microglial activation or chemotaxis. In each analysis, apoE protein and four markers in the latter immune-activation group portended better outcomes.

Discussion: CSF markers of vascular, lipid-metabolic and immune-related functions may explain much of the disjunction between AD biomarker abnormality and symptom severity. In particular, our results suggest the hypothesis that innate immune activation improves cognitive outcomes in persons with AD pathology. This hypothesis should be tested by further study of cognitive outcomes related to CSF markers of innate immune activation.

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Keywords: Alzheimer's disease; Biomarkers; Resilience; Prevention; Immunity

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

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1. Introduction

Forty years after the "rediscovery" of Alzheimer's disease (AD) as the chief cause of old-age dementia [1], we still lack interventions that substantially reduce its morbidity. The resulting AD pandemic creates an imperative to search anew for mechanisms that provoke AD symptoms [2]. Identification of such mechanisms can suggest the development of rational treatments.

Despite enormous information gained, the prevailing hypotheses regarding AD pathogenesis have failed to deliver strategies for mitigation of symptoms. Disappointment is perhaps greatest with respect to the amyloid cascade hypothesis [3]. Substantial evidence supports this conception that oligomerization or aggregation of neurotoxic amyloid β (Aβ) peptides provokes neurodegenerative changes including intraneuronal deposits of hyperphosphorylated and misfolded *tau* protein, leading in turn to synaptic dysfunction [4]. Hopes here were bolstered, but as yet to no avail, by demonstration that much of this change occurs before the onset of cognitive symptoms [5,6], the ideal time for preventive interventions. Especially frustrating has been the finding that anti-amyloid therapies have succeeded in "target engagement," removing or reducing amyloid burden, with little or no symptomatic benefit and even a potential for harm [7–9].

Also popular has been the idea that neurodegeneration in AD results from the deleterious consequences of an innate immune response that accompanies its pathogenesis [10]. This theory was buttressed by epidemiologic findings of reduced AD occurrence in persons with chronic inflammatory disease [11] and in long-term users of nonsteroidal anti-inflammatory drugs [12]. Later observational studies in older patients failed to confirm any advantage of nonsteroidal anti-inflammatory drug use, however [13], and large-scale treatment and prevention trials showed that anti-inflammatory drugs brought no benefit [14] or even possible harm [15–17].

More recently, the genetics of later-onset AD have pointed again to immune as well as lipid metabolic pathways [18], while additional observations have linked AD risk to vascular health [19,20]. For instance, central nervous system (CNS) vascular and blood-brain barrier dysfunction may lead to abnormal accumulation of the AD pathological hallmarks [21]. Altered innate immune functions appear to provoke related changes [22–24], and lipid dysmetabolism is implied by the strong association of AD risk with polymorphisms at apolipoprotein-encoding genes such as *APOE* (especially) and *CLU* (Apo J) [25,26]. Recent data-driven analyses further suggest that changes in some of these pathways occur before accumulation of Aβ abnormality [27,28]. AD therefore appears to represent a failure of several interrelated biological systems. To date, however, investigation of these intertwined pathways has not suggested a route to prevention.

Often overlooked in the search for causes of AD morbidity has been a poorly understood divergence between biological pathogenesis and symptom expression. This "disconnect" is best exemplified by observations that some 20%–30% of older persons whose autopsy results reveal extensive AD pathology were cognitively unimpaired before death [29–31]. Such findings have since been corroborated *in vivo* through positron emission tomography (PET) imaging [32–34]. These and related observations of symptomatic "resilience" [35] suggest that one might suppress the expression of AD dementia and related cognitive symptoms despite the presence of the disease's pathological hallmarks. We suggest that this phenomenon of resilience presents a potential pathway to symptom prevention. Some psychosocial and behavioral antecedents of symptomatic resilience are known [36,37], and other recent work has identified biological factors that may exacerbate or reduce cognitive decline [38–40]. Nonetheless, further elucidation of biological determinants of symptomatic resilience may offer more practical potential pathways for pharmacologic intervention.

To undertake a fresh examination of the last topic, we conducted a series of unbiased searches for biological correlates of symptomatic resilience among 306 participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI-1) who had donated cerebrospinal fluid (CSF). This work relied on an expectation that relevant biological pathways should typically be associated with CSF protein markers of their activity. In previous studies among a subset of this sample, we had identified 23 CSF proteins associated with the AD pathological process [41]. Here, we investigated the latter markers' possible role in symptomatic resilience by assessing their ability to predict cognitive performance and trajectory in relation to a given level of apparent AD pathology.

2. Methods

2.1. Participants

We downloaded data from http://adni.loni.usc.edu. ADNI was launched in 2003 as a public-private partnership led by the principal investigator Michael W. Weiner. Its primary goal has been to test whether serial magnetic resonance imaging, PET, and various clinical, biological, and neuropsychological markers can be combined to measure progression of mild cognitive impairment (MCI) and early AD dementia. We studied 306 ADNI-1 participants across the AD clinical spectrum having available CSF data. These included 90 healthy controls (HC), 147 persons with MCI, and 69 with AD dementia. ADNI assessed the cognitive status of these persons annually using the 11- and 13-point versions of the Alzheimer's Disease Assessment Scale ($ADAS\text{-}Cog_{11}$ and $ADAS\text{-}Cog_{13}$) [42] as well as the Mini-Mental State Examination [43]. Each ADNI site had received approval from its institutional ethical standards committee on human experimentation. Written informed consent was obtained from all research participants and

from collateral informants when applicable. All research complied with ethical principles of the Declaration of Helsinki.

2.2. CSF measurements and classification

The ADNI investigators measured CSF $A\beta_{1\text{-}42}$ and total (t)-*tau* concentrations with research-use-only INNOBIA AlzBio3 immunoassay reagents (Fujirebio, Ghent, Belgium) on an xMap Luminex platform (http://adni.loni.usc.edu/methods/biomarker-analysis/). We assigned the 306 ADNI participants to groups according to their CSF $A\beta_{1\text{-}42}$ and t-*tau* levels, considering them to be "amyloid-positive" if their $A\beta_{1\text{-}42}$ concentrations were below the ADNI-recommended threshold value of 192 pg/mL. Similarly, "*tau*-positive" individuals had t-*tau* values exceeding 93 pg/mL [44]. The ADNI also attempted to assay CSF levels for 159 other proteins using a multiplex X-Map kit from Rules Based Medicine (Myriad RBM, Austin, TX). Rigorous quality control (QC) standards led to exclusion of markers with unacceptable variability in assay results or >10% missing data, yielding only 83 acceptable measures. Results for these were normalized when indicated using Box-Cox or similar transformation techniques (http://adni.loni.usc.edu/wp-content/uploads/2012/01/2011Dec28-Biomarkers-Consortium-Data-Primer-FINAL1.pdf). Our previous studies interrogated these proteins' relation to AD pathology after scoring participants as 0 for no biomarker evidence of such pathology (A−/T−), 1 if positive for amyloid only (A+/T−), or 2 for presence of both amyloid and *tau* abnormalities (A+/T+) [45]. We used Bayes factor analysis to reduce the 83 proteins to 38, showing positive likelihood of association (direct or inverse) with AD biomarker pathology score. Linear regression modeling (with false discovery rate correction, $q \leq 0.05$) then identified the aforementioned 23 "AD-related" proteins (Table 1) that showed a statistically significant association with AD pathology.

2.3. APOE *genotyping*

ADNI *APOE* genotypes had been determined using DNA extracted by Cogenics (Beckman-Coulter, Pasadena, California) [46].

2.4. Analytic methods

2.4.1. Training and validation sets

For purposes of internal validation, we randomly assigned 200 of the noted ADNI participants to a "training" set and the remaining 106 persons to a separate "validation" set. We compared characteristics of the training and validation sets using the Mann-Whitney U test for continuous non-normally distributed variables or the χ^2 test for discrete data when appropriate. Of note, our earlier search for markers associated with AD pathology relied on 74 (70%) of validation set participants (as well as 163, or 63%, of training set participants) who were either HCs or had MCI. To verify lack of circularity in our approach, we therefore identified an alternate, more restricted, set of "AD-related" proteins as before [41], only now omitting consideration of persons in the validation set. The resulting 21 proteins included 19 of the 23 described previously. Substitution of the 21 proteins in the modeling analyses described in the following section produced essentially no change in results.

2.4.2. LASSO regression modeling of baseline cognitive performance

To identify and evaluate markers associated with cognitive performance, we used Least Absolute Shrinkage and Selection Operator (LASSO) regression [47]. This multivariable technique identifies specific variables (items) that predict a given outcome in the context of all others, and assigns optimal item weights for this prediction. Working exclusively in the training set, we developed three LASSO models for prediction of ADNI baseline cognitive impairment score ($ADAS\text{-}Cog_{11}$). Model 1 used only the "classic" AD biomarkers $A\beta_{1\text{-}42}$ and total (t-)*tau*; Model 2 used the 23 AD-related protein markers as described; and Model 3 used the combination of markers in Models 1 and 2. We used a 10-fold cross-validation procedure to optimize penalization and model weight parameters for each model after dividing the training data randomly into ten equal cross-validation sets. On each of ten iterations, we omitted one such set and optimized model weights and penalization parameters for the remaining nine. Averaging marker weights across the ten cross-validation folds then yielded optimal consensus models. We estimated the predictive capacity of these consensus models in the training set by examining predicted versus observed ADAS-Cog scores. Finally, we tested the generalizability of the models by applying them to the never-before-seen validation set. Using a bootstrapping procedure (5000 iterations), we estimated 95% confidence intervals for the proportion of variance (R^2) explained by each model, thereby enabling comparisons of their performance. As a control measure, we tested the specificity of the findings from the 23 AD-related markers (model 2) by comparing their performance with similar "models" obtained using 100 randomly chosen protein marker sets of 23 species from the 60 ADNI-1 CSF proteins that were not "AD-related."

2.4.3. Applying model weights to predict clinical diagnostic category

We then tested whether the marker weights obtained from Model 2 could predict differential expression of symptoms and functional disability, as reflected by ADNI diagnostic category. To do this, we calculated a weighted summary score of marker levels for each participant by multiplying his/her standardized (z-scored) marker levels times the *inverse* of the corresponding marker weights from Model 2 (inverse weighting so that higher score predicted improved

Table 1
List of the 23 "AD-related" markers in ADNI CSF

Markers (with abbreviations)
AXL receptor tyrosine kinase (AXL)
CD40 antigen (CD40a)
Interleukin-3 (IL-3)
Macrophage colony stimulating factor-1 (MCSF-1)
Heparin-binding EGF-like growth factor (HB-EGFL-GF)
Hepatocyte growth factor (HGF)
Transforming growth factor α (TGF-α)
Vascular endothelial growth factor (VEGF)
Heart fatty acid–binding protein (hFABP)
Lectin like oxidized LDL-receptor-1 (LOX-1)
Angiotensin-converting enzyme (ACE)
Tissue factor (TF)
Chromogranin-A (Cg-A)
Cystatin-C
Fibroblast growth factor-4 (FGF-4)
Matrix metalloproteinase-3 (MMP3)
Osteopontin
Tissue inhibitor of metalloproteinases-1 (TIMP-1)
Tumor necrosis factor receptor-2 (TNFR-2)
Vascular cell adhesion molecule-1 (VCAM-1)
Apolipoprotein E (apoE)
Clusterin/apolipoprotein-J (apoJ))
Trefoil factor-3 (TFF-3)

clinical outcomes rather than increased ADAS score). We tested this marker summary score as a predictor of contrasting ADNI diagnostic categories (HC vs. MCI, MCI vs. AD dementia, or HC vs. AD dementia) using multinomial logistic regression. Importantly, we adjusted the regression model not only for age, sex, education, and *APOE* ε4 carrier status, but also for CSF $A\beta_{1\text{-}42}$ and t-*tau* levels. In effect, this adjustment therefore assessed the association of marker summary scores with the various diagnostic categories independent of these covariates. The logistic approach allowed calculation of odds ratios (ORs) for each standardized unit of the weighted protein marker score as a predictor of the three diagnostic contrasts. Upon observing the results (given in the Results section), we evaluated further whether the association of markers with diagnostic assignment was a trivial consequence of cognitive test score variation. To do this, we reran the logistic models, now including $ADAS\text{-}Cog_{11}$ score as an additional covariate.

2.4.4. Using weighted marker scores to predict 4-year cognitive trajectory

Finally, we investigated whether the aforementioned inverse-weighted protein marker summary score from Model 2 predicted subsequent 4-year cognitive trajectory. For this analysis, we used a linear mixed effects analysis to assess the interaction of time with (baseline) weighted marker score as a predictor of ADAS-Cog change. This analysis was adjusted not only for participant age, sex, *APOE* ε4 carrier status, years of education, and CSF $A\beta_{1\text{-}42}$ and t-*tau,* but also for diagnosis and baseline cognitive performance.

All analyses used a two-sided $\alpha = .05$ and relied on MATLAB (MathWorks Inc., Natick, Massachusetts).

2.5. Data availability

All data used for this work are available at the ADNI website (http://adni.loni.usc.edu/) subject to a data usage agreement with the ADNI investigators. Full details can be found at http://adni.loni.usc.edu/data-samples/access-data/.

3. Results

3.1. Demographic characteristics

Demographic characteristics of participants are summarized in Table 2. The training and validation sets were comparable in age, sex ratio, years of education, MMSE, as well as CSF total (t)-*tau* and $A\beta_{1\text{-}42}$. However, there was some disparity in distribution of clinical diagnostic categories, the training set having disproportionate numbers of HCs and fewer AD participants ($\chi^2 = 9.41$, $P = .01$). Presumably owing to its larger proportion of subjects with dementia, the validation set also had somewhat higher (worse) $ADAS\text{-}Cog_{11}$ scores than the training set ($P = .02$). Of note, 34 (38%) HC participants had CSF evidence of Aβ pathology, making them especially suitable candidates for the investigation of symptomatic resilience.

3.2. AD biomarkers' and CSF proteins' relation to cognitive scores

Fig. 1 shows the results of the LASSO modeling approach. Model 1 (AD biomarkers only) demonstrated good predictive accuracy in the training set and generalizability in the never-before-seen validation set, explaining 26% of ADAS score variance in the latter ($P < 10^{-7}$). As expected, t-*tau* had a positive weight (increasing *tau* levels predicting increasing ADAS-Cog score, i.e., greater cognitive deficit), while $A\beta_{1\text{-}42}$ had a negative weight. Model 2, relying exclusively on the 23 AD-related protein markers, explained 31% of variance in validation set cognitive performance ($P < 10^{-9}$), but its apparent improvement over model 1 was uncertain ($P = .19$; Fig. 2). Here, the largest positive weights (strongest association with ADAS score, suggesting diminished cognitive abilities) were observed for heart fatty acid–binding protein, clusterin (apo-J), and hepatocyte growth factor (HGF). The strongest negative weights (lower scores associated with ADAS score or, equivalently, higher scores associated with improved cognition) were apparent for chromogranin-A (Cg-A), apolipoprotein E (apoE), vascular endothelial growth factor (VEGF), and CD-40 antigen (CD-40a). The combination of CSF protein markers and AD biomarkers (model 3) best predicted $ADAS\text{-}Cog_{11}$ scores, explaining 41% of their variance ($P < 10^{-13}$), a

Table 2
ADNI demographics overall and by assignment to training or validation sets

	All participants			Training			Validation			
	HC	MCI	AD	HC	MCI	AD	HC	MCI	AD	P
Sample	90	147	69	69	94	37	21	53	32	**0.01**
Age, mean (s.d.)	75.69 (5.46)	74.99 (7.34)	75.16 (7.60)	76.21 (4.82)	74.90 (7.68)	74.35 (7.99)	73.95 (7.06)	75.15 (6.76)	76.09 (7.13)	0.95
Sex, male:female	46:44	100:47	39:30	34:35	63:31	20:17	12:9	37:16	19:13	0.34
APOE-ε4, Carriers:Non-carriers	68:22	69:78	20:49	54:15	45:49	11:26	14:7	24:29	9:23	0.08
Education years, mean (s.d.)	15.64 (2.94)	15.95 (2.94)	15.16 (2.98)	15.77 (2.94)	15.44 (2.91)	15.03 (3.05)	15.24 (3.00)	16.85 (2.79)	15.31 (2.95)	0.10
CSF $A\beta_{1\text{-}42}$ (pg/mL), mean (s.d.)	207.9 (53.2)	160.5 (50.0)	141.3 (35.6)	205.3 (54.4)	154.3 (44.1)	135.0 (29.3)	216.4 (49.1)	171.5 (57.9)	148.6 (41.0)	0.43
CSF t-*tau* (pg/mL), mean (s.d.)	69.8 (27.7)	105.9 (55.2)	122.9 (60.0)	70.9 (29.1)	110.9 (60.1)	133.2 (67.8)	66.2 (22.9)	97.0 (44.5)	110.9 (47.8)	0.35
Aβ status, negative:positive	56:34	31:116	4:65	42:27	15:79	1:36	14:7	16:37	3:29	0.70
Baseline ADAS-Cog_{11}	6.14 (2.85)	11.90 (4.50)	18.43 (6.78)	6.22 (2.82)	11.91 (4.66)	17.85 (7.92)	5.90 (3.02)	11.84 (4.24)	19.10 (5.21)	**0.02**

NOTE. *P* values are given for overall difference between training and validation sets.
Abbreviations: HC, Healthy controls; MCI, mild cognitive impairment; AD, Alzheimer's dementia; s.d., standard deviation; pg/mL, picograms per milliliter; *APOE-ε4* C:NC, number of *APOE ε4* carriers and noncarriers; CSF, cerebrospinal fluid.

significant improvement over both model 1 and model 2 (P ∼ .01 and .05; Fig. 2). Importantly, excepting HGF, all the key protein markers in model 2 retained similar relevance after addition of the AD biomarkers (model 3), suggesting substantial independence from the latter.

Control "models" were derived from 100 samples of 23 proteins, each drawn randomly from the residue of 60 ADNI proteins unrelated to AD pathology. These failed to converge on an optimal solution in 31% of instances. In the remaining analyses, the randomly chosen protein species typically predicted only a trivial amount of cognitive performance variance in the training set (median $R^2 = 0.02$). Even the best-performing of these control models (training set R^2 up to 0.16) failed to generalize to the validation set (median $R^2 = 0.01$; range = 0–0.10), indicating that these "control protein" models may have reached an "overfitted" state in the training set.

3.3. LASSO protein model weights predict diagnostic category

Results from the adjusted multinomial logistic model for prediction of diagnostic contrasts (HC vs. MCI, HC vs. AD and MCI vs. AD) are presented in the forest plot of Fig. 3. The figure indicates that a 1 standard-unit increase in the inverse-weighted marker summary score from Model 2 was associated in the validation set with a ∼3.5-fold decrease in probability of MCI versus HC (OR = 0.29; 95% confidence interval [CI] = 0.12–0.68, $P < .005$). Similarly, each unit increase in marker score implied a 4.1-fold decrease in the probability of AD dementia versus MCI (OR = 0.25; 95% CI = 0.11–0.55, $P < .001$) and a 14.2-fold decrease in the probability of AD dementia versus HC (OR = 0.07; 95% CI = 0.02–0.22, $P < .001$). These results were only partly mitigated by inclusion of cognitive scores in the model (all P remaining < 0.01), suggesting that the markers predicted functional capacity (important to the described diagnoses) beyond pure cognitive deficit.

3.4. Weighted marker score predicts rate of 4-year cognitive decline

Here, we tested the association between the weighted protein marker score from Model 2 and subsequent four-year cognitive trajectory, verifying that such association would survive adjustment not only for biomarkers and AD risk factors but also for baseline cognitive performance and clinical diagnostic category. The fully adjusted analysis indicated that each standard unit increase in inverse-weighted marker score was associated with a decrease in slope of ADAS-Cog_{11} performance of β = 0.87 points/year, (standard error = 0.39; $P = .04$; Fig. 4). The limited sample size of the validation set precluded evaluation of weighted marker score prediction of 4-year cognitive trajectory among individual diagnostic categories.

4. Discussion

We examined CSF AD-related protein markers alongside biomarkers of AD for their relevance to cognitive symptom expression. To do this, we used a 200-person training set of participants from ADNI-1, applying LASSO regression with a ten-fold cross-validation procedure to assign predictor variable weights. The three resulting models were then tested in a validation set of the remaining 106 ADNI participants. We found that the AD biomarker and CSF protein models appeared to be additive, with the contributions of their constituent variables being mostly independent. Lack of collinearity between the two sets of markers was further

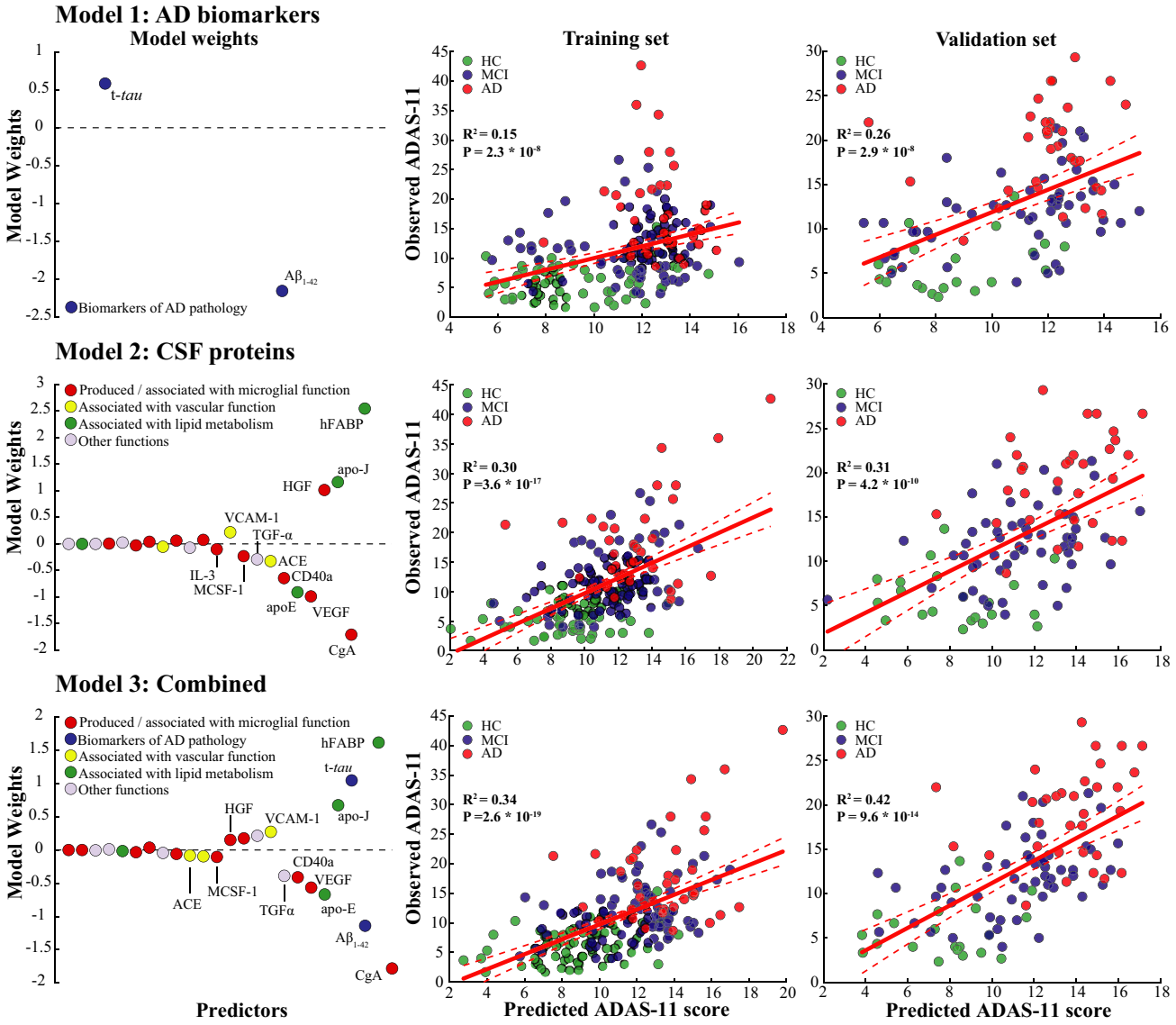

Fig. 1. LASSO regression modeling for prediction of $ADAS_{Cog}$-11 performance. Three models were trained to predict baseline $ADAS_{Cog}$-11 performance. Model 1 (top row) considered typical AD biomarkers (CSF $A\beta_{1-42}$ and t-tau) only. This model's predictions explained only a fraction of the variance in training set $ADAS_{Cog}$-11 scores (R^2 = 0.15) but generalized well to the validation set (R^2 = 0.26). Model 2 (middle row) considered the 23 CSF AD-related proteins of interest here. This model relied strongly on hFABP, apoJ, HGF, Cg-A, apoE, VEFG, and CD-40a (color-coded with relevant functions annotated in the figure), which apparently accounted for >90% of its predictive abilities. It provided robust predictions in both the training (R^2 = 0.30) and validation sets (R^2 = 0.31). Model 3 (bottom row) used the combination of AD biomarkers and the 23 CSF proteins. It provided good to excellent predictions in the training (R^2 = 0.36) and validation sets (R^2 = 0.42). Dots in the left column represent marker weights in each model. The middle column shows correlations in the training set between observed $ADAS_{Cog}$-11 scores and those predicted by each model, with color-coded dots indicating each participant's ADNI diagnosis. The right hand column shows equivalent correlations for the validation set. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; HC, healthy control; CSF, cerebrospinal fluid; hFABP, heart fatty acid–binding protein; HGF, hepatocyte growth factor; VCAM, vascular cell adhesion molecule-1; TGF-α, transforming growth factor α; ACE, angiotensin-converting enzyme; IL, interleukin; MCSF-1, macrophage colony stimulating factor-1; VEGF, vascular endothelial growth factor; ADAS, Alzheimer's Disease Assessment Scale; apoE, apolipoprotein E protein.

suggested by the relative invariance of protein marker weights in the conjoint Model 3 versus that derived from the proteins only (Model 2).

In particular, the protein marker Model 2 predicted disjunction between degree of AD pathology and symptom expression. Not only did it predict (cross-sectional) cognitive performance in the never-before-seen validation set, a summary score derived from its constituent marker weights also provided strong prediction of ADNI clinical diagnostic category. Importantly, the latter prediction survived statistical adjustment for CSF levels of Aβ and *tau* as well as AD risk factors and, notably, cognitive score.

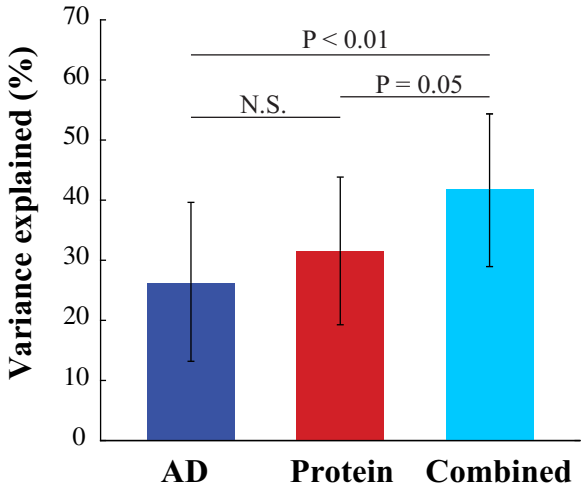

Fig. 2. Statistical comparison of models trained to predict baseline $ADAS_{Cog}$-11 performance. Each model's performance was evaluated in the unseen validation set. Bars represent proportion of variance explained ($R^2 \times 100$) for each model. We generated 95% confidence intervals using bootstrapping (5000 iterations) and used a bootstrap test to compare models. Model 1: typical AD biomarkers; model 2: 23 AD-related proteins; model 3: combined AD biomarkers and AD-related protein markers. Abbreviations: N.S., Not significant; AD, Alzheimer's disease.

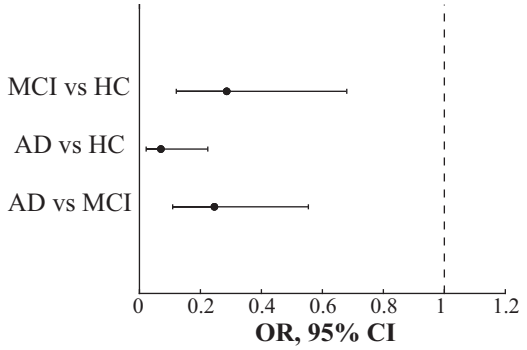

Fig. 3. Weighted Model 2 marker summary score predicts contrasts among clinical diagnostic categories. A multinomial logistic regression model evaluated association of weighted protein marker summary scores with contrasts between clinical diagnostic groups in the validation set. The odds ratios (ORs, point estimate indicated by dots, with 95% CI shown by horizontal lines) show decreased likelihood of a more severe diagnosis (AD dementia vs. MCI vs. HC) after adjustment for age, sex, *APOE* ε4 carrier status, and years of education. ORs suggest change associated with each standardized unit increase in weighted marker score. Importantly, the model was also adjusted for CSF levels of $A\beta_{1\text{-}42}$ and t-*tau*, indicating distinction in clinical severity for a given level of AD pathology. Abbreviations: MCI, Mild cognitive impairment; HC, healthy control; AD, Alzheimer's disease; CI, confidence interval.

Otherwise stated, the protein markers alone predicted variation in diagnosis for a given level of AD pathology and cognitive score. In fully adjusted models that also included a term for baseline cognitive score and diagnosis, the inverse-weighted protein marker summary score also predicted rate of cognitive change over the succeeding four years. Together, these observations suggest that AD-related protein markers are strongly associated with baseline cognition, with cognitive diagnosis, and with cognitive decline associated with a given level of AD biomarker "pathology." Limitation of this effect to the 23 AD-associated markers was suggested by the absence of any similar prediction of cognitive outcomes in models constructed from multiple sets of 23 "control" markers from the remaining 60 ADNI-1 protein species.

4.1. Function of the predictive CSF proteins

While these findings await independent confirmation, we suggest it is reasonable here to consider mechanistic explanations that might advance our understanding of symptomatic resilience to AD pathology. Notably, most of the predictive capability of the 23 AD-related proteins resided in ten marker species that were responsible for >95% of the observed effect (data not shown). In models that included (were adjusted for) Aβ and *tau* levels, these were fatty acid–binding protein (FABP), apoJ, apoE, angiotensin-converting enzyme, Cg-A, CD-40a, VEGF, HGF, Transforming Growth Factor α, and macrophage colony stimulating factor-1. Among these, only HGF appeared to be collinear with the AD biomarkers, becoming inapparent as a predictor of cognitive outcomes when analyses included the biomarkers. A gene ontology analysis suggested that the nine remaining proteins are involved in a variety of overlapping mechanisms involved in lipid metabolic, immune, and vascular pathways.

The functions of several of these proteins recall findings from the genetics of late-onset AD. For example, apoE protein (determined by *APOE* genotype, the strongest genetic risk factor for late-onset AD after age) was among the nine key protein markers. ApoJ (clusterin), conditioned by the *CLU* risk polymorphism [26], was another key "predictor." Both apoE and apoJ appear to be involved in a dynamic equilibrium between Aβ plaques and soluble Aβ species, possibly owing to their role in cholesterol transport [48] (for apoE) or in breakdown of protein aggregates (for apoJ) [49]. ApoE also has an important role in astrocyte-mediated clearance of Aβ [50]. Recent data suggest it may also be important in microglial activation in neurodegenerative diseases via coupling with the triggering receptor expressed on myeloid cells 2 (TREM-2) pathway [51], the product of another important AD risk gene. FABP is also implicated in the transport of lipids and may be elevated in the CSF of patients with AD and individuals with progressive MCI [52].

FABP-mediated lipid metabolism may also be linked to inflammatory processes [53], in keeping with a broader notion that CNS lipid metabolism is important to innate immune activation. It is probably not surprising, therefore, that the aforementioned three proteins conjoin

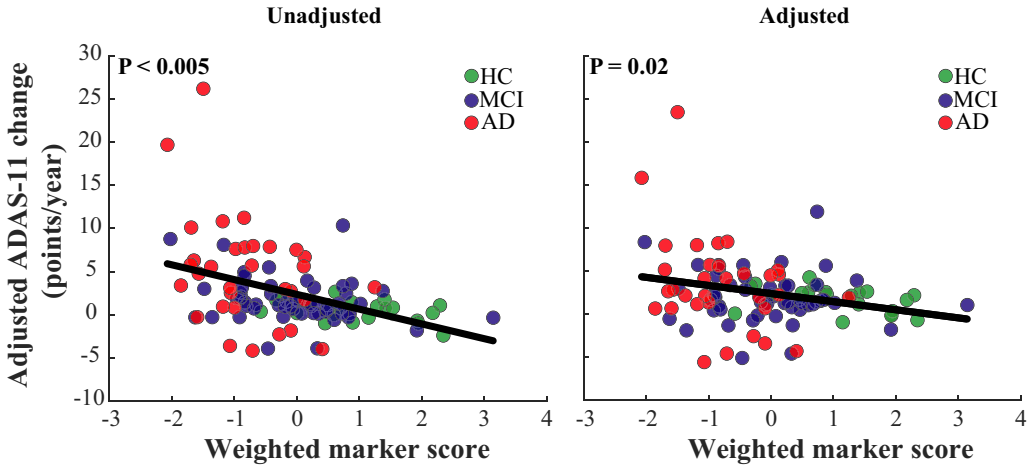

Fig. 4. Weighted protein marker summary score from Model 2 predicts 4-year cognitive trajectory. Linear mixed-effects analyses were used to evaluate association of weighted protein marker summary scores with 4-year trajectory on the $ADAS_{Cog}$-11 scale. These analyses were adjusted for age, sex, *APOE* ε4 carrier status, years of education, CSF t-*tau*, and Aβ levels (left). Higher summary scores were associated with a decrease in $ADAS_{Cog}$-11 scores ($P < .005$). As expected, this apparent relationship was diminished moderately with adjustment for baseline cognitive performance (right), but summary scores remained robustly associated with longitudinal cognitive decline ($P < .02$). Abbreviations: MCI, Mild cognitive impairment; HC, healthy control; AD, Alzheimer's disease; ADAS, Alzheimer's Disease Assessment Scale.

with four other evidently immune-related markers among the "short list" of nine proteins that appear to modify clinical outcomes. Specifically, CD-40a is a key mediator of immune activation believed to hold an early role in the pathogenesis of AD [54,55]. It activates antigen presenting T cells while it regulates the deposition of Aβ. Cg-A is similarly associated with CNS microglial activation [56,57]. Although important to vascular homeostasis [58], VEGF has been shown also to induce microglial chemotaxis and proliferation [59,60] and to be an important mediator of the immune response to tumors [61]. Macrophage colony stimulating factor-1 causes proliferation and differentiation of macrophages, regulates number of microglial cells and their state of differentiation, and promotes neuronal survival [62,63].

Notably, each of the aforementioned four "immune-related" markers was inversely related to ADAS-Cog score, that is, higher levels predicted improved cognition. Because there has been a widespread assumption that "inflammatory" processes exert a deleterious influence on AD symptom development (see Section 4.3 below), we had expected higher levels of these four markers to be associated with increasing symptom severity, but we found the opposite to be true. This observation appears to converge with other evidence that some enhanced immune responses can be beneficial for individuals who are older [64] or who have established AD dementia [65]. Therefore, the described functions of the predictive markers may suggest that immune activation occurs as a response to insults such as AD pathology, presumably involving activation and recruitment of immune cells. Such activation could, in some instances, enhance clearance of toxic proteins and promotion of cell survival, thereby resulting in improved cognitive outcomes for a given level of AD pathology.

4.2. *Strengths and limitations*

The principal strength of this work appears to be its reliance on an unbiased data-driven, hypothesis-free approach to identify markers associated with both AD pathology and symptom expression. Our analytical plan was based on rigorous cross-validation and internal validation procedures to build models from important predictors that generalized well to unseen data.

However, several limitations should also be noted. Most importantly, these analyses relied on data from a protein screen of ADNI CSF that was assayed using multiplex technology. The ADNI investigators had initially attempted to assay 159 proteins, only 83 of which passed quality control criteria. Such results occur typically because of insufficient assay sensitivity for many analytes, here including several key markers of immune activation. Furthermore, despite their apparent links to immune activation, the predictive proteins act at the interface of several processes thought to be key in the AD cascade. It remains possible, therefore, that other pathways may act independently or in concert with immune activation to modulate, and possibly improve, cognitive function. A further limitation of our analyses was their reliance principally on cross-sectional data, limiting our ability to infer ordinality or causality of the observed associations. Finally, we relied on CSF Aβ and *tau* as indicators of the extent of AD pathology (an important component in the phenomenon of resilience). Unfortunately, we could not include PET measures of Aβ or *tau* pathology because few

participants had these measures available (96 for Aβ, 19 for *tau*), many with an excessive interval between baseline and PET imaging (median lag from LP to scan: 60 months for Aβ, 132 months for *tau*). Similarly, cortical thickness data from a region of interest, as described by Jack et al. [66], or hippocampal volume as measures of AD progression also resulted in significant missing data (∼20%), although these yielded similar results in reduced samples (data not shown). Before their significance can be fully evaluated, therefore, our results require corroboration in other data sets, preferably using assay methods with improved sensitivity as well as other indicators of AD pathology.

Nevertheless, the available data suggest an important pathway for modification, and possible amelioration, of symptomatic expression of AD and therefore a hypothesis for future investigation.

4.3. A hypothesis and experimental approaches to its assessment

Hypothesis: Immune activation modulates the cognitive and functional deficits that otherwise accompany the accrual of "classical" AD pathology.

It has long been understood that immune activation accompanies evidence of the AD process [67]. Early epidemiological studies showed an inverse association between anti-inflammatory drug use and AD risk [12,68]. These findings led to widespread speculation that AD pathology induced immune activation, which in turn generated a neurotoxic environment, neuronal death, and eventually dementia [69]. This formulation recalls other instances in which uncontrolled CNS immune responses lead to nervous system damage and cognitive impairment (e.g., in Lyme disease) [70]. In neurodegenerative disease models, maladaptive immune responses appear to provoke neuronal death through the generation of reactive astrocytes [71]. The present results are at odds with these ideas, however, suggesting instead that some components of innate immune activation may be associated with improved cognitive outcomes in AD. This improvement might result from increased clearance of Aβ species [72], a process in which astrocytes appear also to play an important role [50].

Importantly, most CNS immune responses are provided by microglia and astrocytes. These cells have multiple functions and roles at the interplay of pathways thought to be involved in AD pathogenesis. For instance, astrocytes are the main producers of apoE protein in the CNS and are clearly involved in blood brain barrier (BBB) function and in the clearance of Aβ and neurotoxic neurotransmitters [73]. In addition, both astrocytes and microglia help maintain CNS homeostasis and provide trophic support to neurons. While malfunction of these important cells may result from accumulating AD pathology, they may also be the cause of such pathology by provoking either accumulation of pathological proteins, BBB dysfunction, or deterioration and loss of trophic support. Any of these may promote neurodegeneration. In this context, it appears noteworthy that risk alleles at the polymorphic TREM-2 and CD-33 genetic loci are associated with reduced microglial clearance of Aβ plaques [22,74,75]. These loss-of-function mutations result in reduced microglial activation and clustering around plaques [76].

An important limitation of the latter observations, however, is that they derive mainly from animal models. We know of no present evidence of similar mechanisms in human studies, notably because these efforts have encountered difficulties in reliable measurement of the most important markers of immune activation in CSF [77,78]. Thus, to date, human studies of association between disease and fluid markers of inflammation have yielded contrasting results [79].

While we and others have observed reduced CSF immune marker levels in individuals with evidence of "pure" amyloid pathology [41,80], (thereby suggesting that a maladaptive immune response might result in brain accrual of Aβ) the noted limitations in measurement sensitivity restricted our analysis to only 23 markers. Measurement of many other protein markers, particularly other markers of immune activity, could render a more complete analysis of biological networks involved. Future efforts to test the proposed hypothesis and identify its related pathways will therefore require newer immune marker assay methods having improved sensitivity. Although costly, these techniques should allow identification and measurement of many more relevant immune marker proteins.

As here, an expanded set of envisioned assay results would benefit from the use of unbiased, data-driven feature selection approaches. Importantly, these techniques identify and assess the relevance of individual markers in the context of all others. An important objective of the proposed work should be to identify which among many available markers of immune activity have the greatest apparent "effect" on cognitive outcomes—a topic not readily studied in animal models of preclinical AD. These markers, or families of them, can in turn suggest specific immune pathways relevant to the phenomenon of resilience, thereby prompting additional focused biological investigation using modern, high-resolution techniques.

Finally, we would note that our current or proposed analyses relate to cross-sectional data and therefore cannot assess important questions about the ordinality and potential causality of discovered associations. To answer these last questions, longitudinal data will be needed. Such data could in turn be analyzed using recently introduced machine-learning algorithms that can provide a virtual

"motion picture" of sequential events in the biological pathways underlying resilience. Such work may also identify immune pathways involved in accumulation or clearance of pathological proteins and symptomatic resilience, thereby pointing more directly to timing at which pathways could be either upregulated or downregulated to achieve symptom mitigation. Together with other biological or mechanistic experimentation mentioned previously, results from such studies may suggest promising new targets for novel prevention strategies.

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RESEARCH IN CONTEXT

1. Systematic review: To date, most studies on symptomatic resilience to Alzheimer's disease (AD) pathology have investigated lifestyle and psychosocial phenomena. Little prior work has investigated biological pathways that may deter symptom expression.
2. Interpretation: Data-driven analyses suggest that vascular, lipid metabolic, and, especially, immune pathways explain much of the disjunction between classic AD biomarker abnormality and symptom severity. In keeping with new observations reported here, we propose a hypothesis that activation of innate immune pathways, revealed by CSF markers, can improve cognitive outcomes in persons developing AD pathology.
3. Future directions: We propose a research framework to test the foregoing hypothesis. This research should include assessment of previously unmeasurable immune markers through use of new high-sensitivity bioassays. Longitudinal data on such markers along with AD biomarkers and cognitive measures can be analyzed using cutting-edge unsupervized machine learning techniques. Such analyses may suggest intervention strategies for prevention of AD symptoms.

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