Original Investigation

Combined Plasma and Cerebrospinal Fluid Signature for the Prediction of Midterm Progression From Mild Cognitive Impairment to Alzheimer Disease

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IMPORTANCE A reliable method of detecting Alzheimer disease (AD) in its prodromal state is needed for patient stratification in clinical trials or for personalizing existing or potential upcoming therapies. Current cerebrospinal fluid (CSF)– or imaging-based single biomarkers for AD offer reliable identification of patients with underlying AD but insufficient prediction of the rate of AD progression.

OBJECTIVE To optimize prediction of progression from mild cognitive impairment (MCI) to AD dementia by combining information from diverse patient variables.

DESIGN, SETTING, AND PARTICIPANTS This cohort study from the Alzheimer Disease Neuroimaging Initiative (ADNI) enrolled 928 patients with MCI at baseline and 249 selected variables available in the ADNI data set. Variables included clinical and demographic data, cognitive scores, magnetic resonance imaging–based brain volumetric data, the apolipoprotein E (*APOE*) and translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) genotypes, and analyte levels measured in the CSF and plasma. Data were collected in July 2012 and analyzed from July 1, 2012, to June 1, 2015.

MAIN OUTCOMES AND MEASURES Progression from MCI to AD within 1 to 6 years. To determine whether combinations of markers could predict progression from MCI to AD within 1 to 6 years, the elastic net algorithm was used in an iterative resampling of a training- and test-based variable selection and modeling approach.

RESULTS Among the 928 patients with MCI in the ADNI database, 94 had 224 of the required variables available for the modeling. The results showed the contributions of age, Clinical Dementia Rating Sum of Boxes composite test score, hippocampal volume, and multiple plasma and CSF factors in modeling progression to AD. A combination of apolipoprotein A-II and cortisol levels in plasma and fibroblast growth factor 4, heart-type fatty acid binding protein, calcitonin, and tumor necrosis factor–related apoptosis-inducing ligand receptor 3 (TRAIL-R3) in CSF allowed for reliable prediction of disease status 3 years from the time of sample collection (80% classification accuracy, 88% sensitivity, and 70% specificity).

CONCLUSIONS AND RELEVANCE These study findings suggest that a combination of markers measured in plasma and CSF, distinct from β-amyloid and tau, could prove useful in predicting midterm progression from MCI to AD dementia. Such a large-scale, multivariable-based analytical approach could be applied to other similar large data sets involving AD and beyond.

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The use of biomarkers for early diagnosis of Alzheimer disease (AD) has been investigated widely, and several studies showed that cognitively normal individuals who later develop AD dementia can be identified earlier in the disease course by use of imaging and cerebrospinal fluid (CSF) biomarkers.[1,2] Levels of β-amyloid (Aβ) in the brain demonstrate the earliest AD-type changes[3] and can be detected by measuring CSF levels of Aβ42 or using positron emission tomography (PET). Unfortunately, the temporal resolution of Aβ-based biomarkers is too weak to accurately predict progression of the disease course from mild cognitive impairment (MCI) to AD dementia.

Alzheimer disease can be represented by a continuum from cognitively normal-appearing individuals with evidence of accumulation of Aβ in the brain to those with severe dementia.[4,5] Mild cognitive impairment is an intermediate stage between normal cognitive decline with aging and dementia; during this stage, patients have a greater cognitive decline than expected for their age and educational level.[6-8] Current data indicate that rates of MCI progression to AD are estimated at approximately 10% per year[9] and represent the population with the highest risks for progression to AD.[10] Other causes of MCI include dementia with Lewy bodies, Parkinson disease, frontotemporal dementia, and stroke.[6] Owing to the heterogeneity of the causes of MCI, research criteria and biomarkers were defined; according to the most recently published diagnostic criteria,[6] neurodegeneration and cerebral amyloidosis are necessary to determine which individual with MCI has underlying AD. In the search for diagnostic and prognostic biomarkers of AD, the field has focused heavily on CSF[11] and neuroimaging markers.[12] For example, CSF levels of Aβ42[11] or Aβ levels on PET imaging[13] are both indicative of cerebral amyloidosis, and magnetic resonance imaging (MRI), fludeoxyglucose F 18–labeled (FDG)-PET,[12] or CSF levels of t-tau[11] are usually used to assess neurodegeneration. Although several studies showed that progression of individuals with MCI to dementia within a few years could be predicted by MRI findings,[14,15] FDG-PET,[16] or CSF examination,[17] no unified or combination of biomarkers has emerged to predict the time to progression.

Herein, we used data from the Alzheimer's Disease Neuroimaging Initiative (ADNI), which is an ongoing, longitudinal, multicenter study.[18] In this data set, we identified combinations of specific individual patient variables that predict progression from MCI to AD 1 to 6 years before the clinical diagnosis of AD.

Methods

Patient Data

Data used in preparing this article were produced by ADNI (eMethods in the Supplement) and were obtained in July 2012. A complete list of the 249 individual patient variables used for the analysis is provided in the **Table**.[19-25] A summary of patient variables among the 928 study participants with MCI are available in eTable 1 in the Supplement. Data preprocessing is detailed in the eMethods in the Supplement. The individual baseline patient variables used in this study were only available for participants in ADNI acquired until 2010. For the multivariable analysis to model progression to AD with baseline data, 94 patients with MCI had all 224 required variables (**Figure 1**A). The number of patients with MCI included in the follow-up declined during the 6 years (eFigure 1 in the Supplement). In the most recent version of the ADNI database, 24 of the 94 patients with MCI had a longer follow-up. The ADNI study was approved by institutional review boards of all participating institutions. Informed written consent was obtained from all participants at each site.

Utility of Established Biomarkers of AD

To test the utility of the established AD CSF biomarkers Aβ42, t-tau, and p-tau to predict progression from MCI to AD in the ADNI cohort, we used these variables to model the binary end point of stable MCI vs progression to AD within 1 to 6 years (progression was defined by an on-site physician). We calculated a receiver operating characteristic curve using the R pROC package.[26] We computed 95% CIs for the area under the curve using the approach of DeLong et al.[27] Not all 928 participants underwent lumbar puncture, and the exact sample size available for each CSF biomarker and progression time point is shown in eTable 2 in the Supplement.

Visualization of the Associations Among Large Panels of Variables

To depict associations within and between the 8 categories of variables, a circular visualization of the correlation plot was generated using the qgraph package for R.[28] This plot is based on calculating pairwise rank correlations between complete observations. The plot displays a network with nodes representing the variables and edges linking any pairs of variables based on their correlation coefficient with each other. A threshold of $|r| > 0.3$ was used to display only the strongest correlations. The circular visualization of the correlation plot includes all 249 variables and data available from 928 patients.

Selection and Combination of Specific Individual Patient Variables

Prediction of progression from MCI to AD used 224 variables in only 94 of the 928 patients because data were missing for most of the patients (Figure 1A, Table, and eMethods and eTable 3 in the Supplement). To identify variables associated with progression from MCI to AD within 1 to 6 years, we used an elastic net algorithm[29,30] in an iterative resampling of a training- and test-based variable selection and modeling approach. Briefly, elastic net was applied on the training subset to select variables that best discriminate between patients with stable and progressive MCI. The quality of each model was estimated on the test data set using the classification accuracy rate, sensitivity, specificity, and stability. One thousand resamplings of the learning and test data sets were performed, and the variables were ranked according to their number of appearances across permutations in the elastic net models to select the top variables. To refine these predictive models to a simpler final model, we then used a forward classification strategy and compared our results with those obtained by chance.

Table. Individual Patient Variables Included in the Study

Category by Index	Variable
Clinical and demographic characteristics	
1	Sex[a]
2	No. of years of education
3	Age at enrollment[a]
Cognitive scores	
4	MMSE score[a,b]
5	ADAS total score[a,c]
6	ADAS modified[a,c]
7	CDR composite test score[a,d]
8	CDR-SOB composite test score[a,d]
9	FAQ[e]
10	GDS[f]
11	Modified Hachinski Ischemia Scale score[g]
12	NIQ Total score[h]
MRI-based brain regional volumes	
13	Brain volume
14	EICV
15	Ventricular volume normalized by EICV[a]
16	Hippocampal volume normalized by EICV[a]
17	Inferior lateral ventricular volume normalized by EICV[a]
18	Middle temporal volume normalized by EICV[a]
19	Inferior temporal volume normalized by EICV[a]
20	Fusiform cortical volume normalized by EICV[a]
21	Entorhinal cortical volume normalized by EICV[a]
Genetic	
22	*APOE4* allele carrier[a]
23	No. of *APOE4* alleles
24	*TOMM40* PolyT variable-length polymorphism allele 1
25	*TOMM40* PolyT variable-length polymorphism allele 2
Fluid variables	
26	8-Iso-$PGF_{2\alpha}$
27	8,12-iso-iPF2α
28	CSF white blood cell count
29	CSF red blood cell count
30	CSF total protein concentration
31	CSF glucose level
32	Total plasma homocysteine level
33	Plasma Aβ40 level
34	Plasma Aβ42 level
35	Plasma Aβ40:Aβ42 ratio
Established AD CSF biomarkers	
36	CSF Aβ42 level[a]
37	CSF t-tau level[a]
38	CSF p-tau level[a]
39	CSF Aβ42 to t-tau ratio
40	CSF Aβ42 to p-tau ratio
41	CSF p-tau to t-tau ratio
Subset of CSF communicome[i]	
42 to 115	74 CSF analytes measured by multiplex assay (among 159 measured)[a]
Subset of plasma communicome[i]	
116 to 249	134 Plasma analytes measured by multiplex assay (among 190 measured)[a]

Abbreviations: Aβ, β-amyloid; ADAS, Alzheimer Disease (AD) Assessment Scale; *APOE4*, apolipoprotein ε4; CDR, Clinical Dementia Rating scale; CSF, cerebrospinal fluid; EICV, estimated intracranial volume; FAQ, Functional Assessment Questionnaire; GDS, Geriatric Depression Scale; iPF2α, isoprostane F2α; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; NIQ, Neuropsychiatric Inventory Q; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; SOB, Sum of Boxes; *TOMM40*, translocase of outer mitochondrial membrane 40 homolog.

[a] Variable included in the modeling of progression from MCI to AD.

[b] Folstein et al.[19]

[c] Rosen et al.[20]

[d] Morris.[21]

[e] Pfeffer et al.[22]

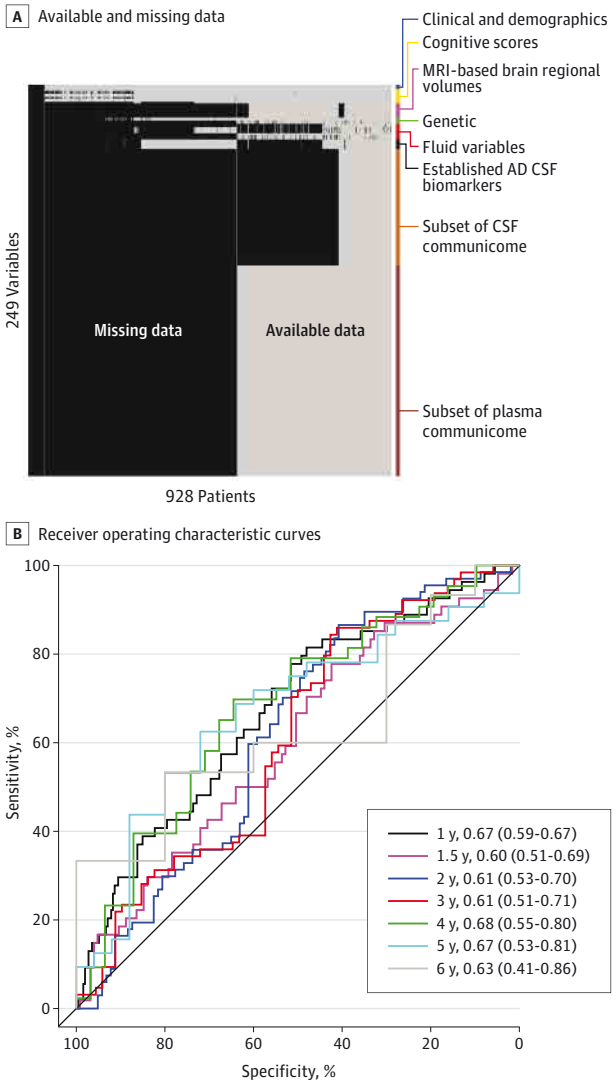
[f] Sheikh and Yesavage.[23]

[g] Rosen et al.[24]

[h] Kaufer et al.[25]

[i] Indicates the subset of the secreted proteome that cells use to communicate with each other, measured with Luminex assays (Myriad RBM).

Figure 1. Initial Analysis of the Data Available for 928 Patients With Mild Cognitive Impairment (MCI)



A, Overview of the 249 variables (listed in the Table and eTable 3 in the Supplement) is given in 8 categories. B, Receiver operating characteristic curves combine cerebrospinal fluid (CSF) β-amyloid 42 (Aβ42), t-tau, and p-tau in modeling progression from MCI to Alzheimer disease (AD) within 1 to 6 years. The areas under the curve (AUCs) (95% CI) are given for each year. Receiver operating characteristic curves for Aβ42, t-tau, and p-tau separately are available in eFigure 2 in the Supplement; the sample size available is shown in eTable 2 in the Supplement. Diagonal line indicates completely random discrimination; MRI, magnetic resonance imaging.

Data were analyzed from July 1, 2012, to June 1, 2015. A complete description of the approach used is detailed in the eMethods in the Supplement.

Results

Utility of Established Biomarkers of AD to Predict Progression to AD

First, we evaluated the utility of CSF levels of Aβ42, t-tau, and p-tau to predict progression to AD within 1 to 6 years and performed a receiver operating characteristic analysis. These established diagnostic AD biomarkers combined (Figure 1B) or separately (eFigure 2 in the Supplement) cannot be used for reliable prediction of progression from MCI to AD. Hence, exploring other fluid markers and clinical variables is indicated for this purpose.

Associations Among Large Panels of Variables

To get an overview of the associations among the 249 variables available across 928 patients with MCI from the ADNI database (Table), we generated a circular visualization of correlation plot (**Figure 2**). This plot revealed complex relationships within and between groups of variables. For instance, concentrations of secreted proteins involved in intercellular communication (previously termed the *communicome*[31]) measured in plasma or CSF samples were strongly correlated within and between these 2 body fluids. Age and sex were correlated with the communicome but not with the established CSF biomarkers Aβ42 and t-tau. In contrast, the apolipoprotein E (*APOE*) genotype was linked to established CSF biomarkers but not to the communicome. Proteins of the communicome are linked differentially with established AD CSF biomarkers and MRI-based brain volumes, which suggests that variables from different categories could carry complementary information about the disease. This information may help to predict progression from MCI to AD.

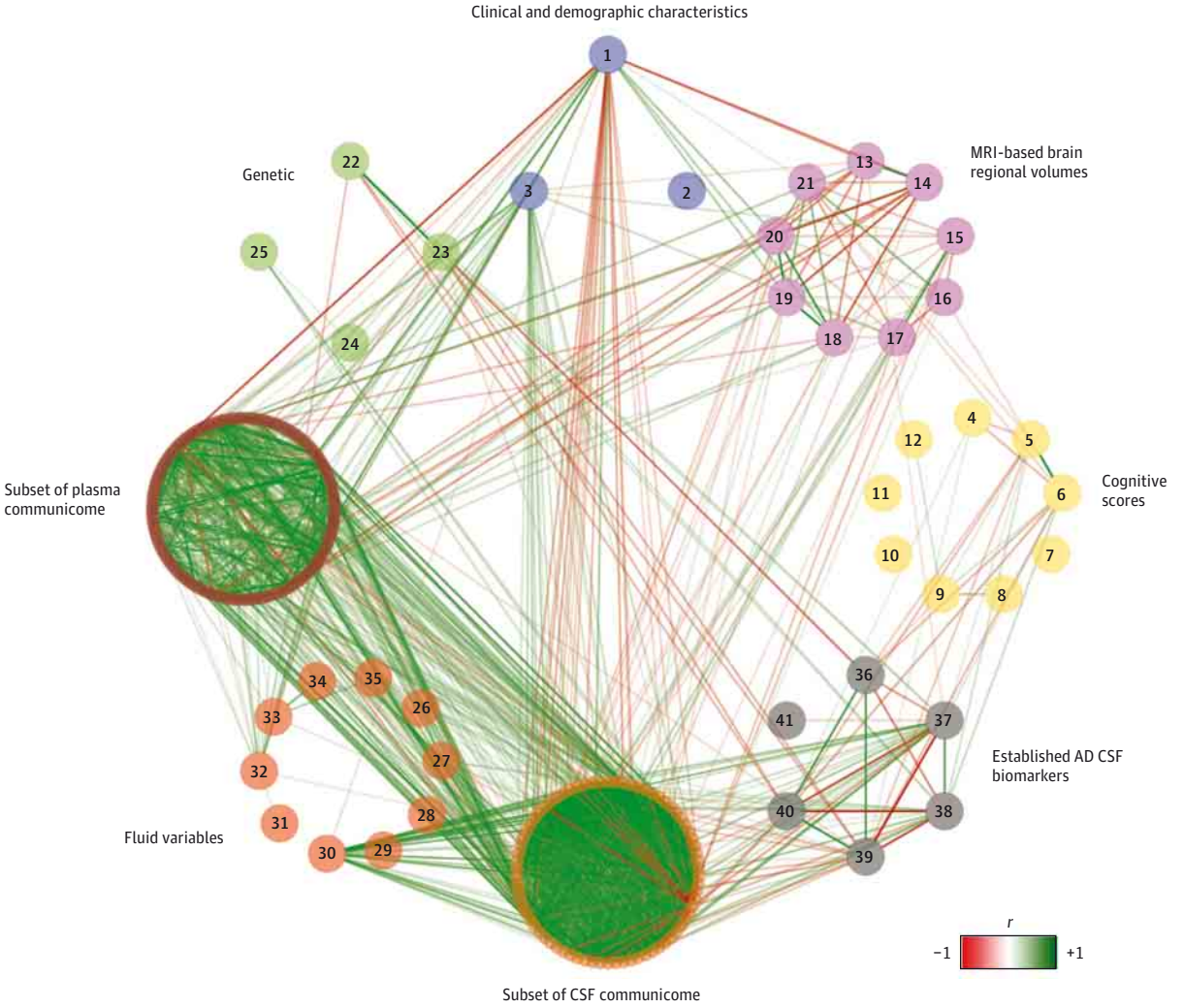
Prediction of Progression From MCI to AD Within 1 to 6 Years

To determine whether combinations of markers could predict progression from MCI to AD within 1 to 6 years, we had to restrict our analysis to 94 patients with MCI and 224 variables with sufficient available baseline data (Table and eTable 3 in the Supplement). Of these 94 patients, a growing number had progression to AD within 1 to 6 years, whereas the follow-up of individuals dropped below 50% after 4 years (eFigure 1 in the Supplement; cohort characteristics are given in eTable 4 in the Supplement).

We built predictive models for each progression time point in 6- or 12-month increments (**Figure 3**A and eMethods in the Supplement); for each model we calculated sensitivity and specificity using the top 2 to 20 selected variables (Figure 3B). Among all models that used 20 variables, those predicting 2 or 3 years were most accurate (sensitivity/specificity, 76%/70% for 2 years; 87%/73% for 3 years), whereas the other models had low sensitivity (for 1, 1.5, and 6 years) or low specificity (for 4 and 5 years). As indicated by the SD across permutations, prediction of progression within 4 years or later was less stable than earlier prediction of progression to AD (Figure 3C).

A total of 80 of 224 variables were selected at least once as one of the top 20 variables in the different models (eTable 5 in the Supplement). The most frequently used top variable was the neuropsychometric Clinical Dementia Rating Sum of Boxes composite test score (CDR SOB), which was included in 6 of 7 models. The molecular marker CSF tumor necrosis factor–related apoptosis-inducing ligand receptor 3 (TRAIL-R3) was included in 5 models, plasma apolipoprotein A-II (ApoA-II) and

Figure 2. Associations Among 249 Variables Shown by a Circular Visualization of Correlation (CVC) Plot



Includes 928 patients with mild cognitive impairment. Most of the variables were unavailable for the 928 patients, as indicated in Figure 1A. We calculated the correlation coefficient between pairwise complete observations. Lines (edges) represent the Spearman rank correlation coefficient (*r*) between 2 variables (nodes). Variables were grouped in 8 categories. Category names of the variables are indicated; except for the cerebrospinal fluid (CSF) and plasma communicome categories, the 249 variables are labeled by numbers (more details on categories and variables are given in the Table and eTable 3 in the Supplement). The CVC plot was thresholded at |*r*| > 0.3 to display only the strongest correlations. AD indicates Alzheimer disease; MRI, magnetic resonance imaging.

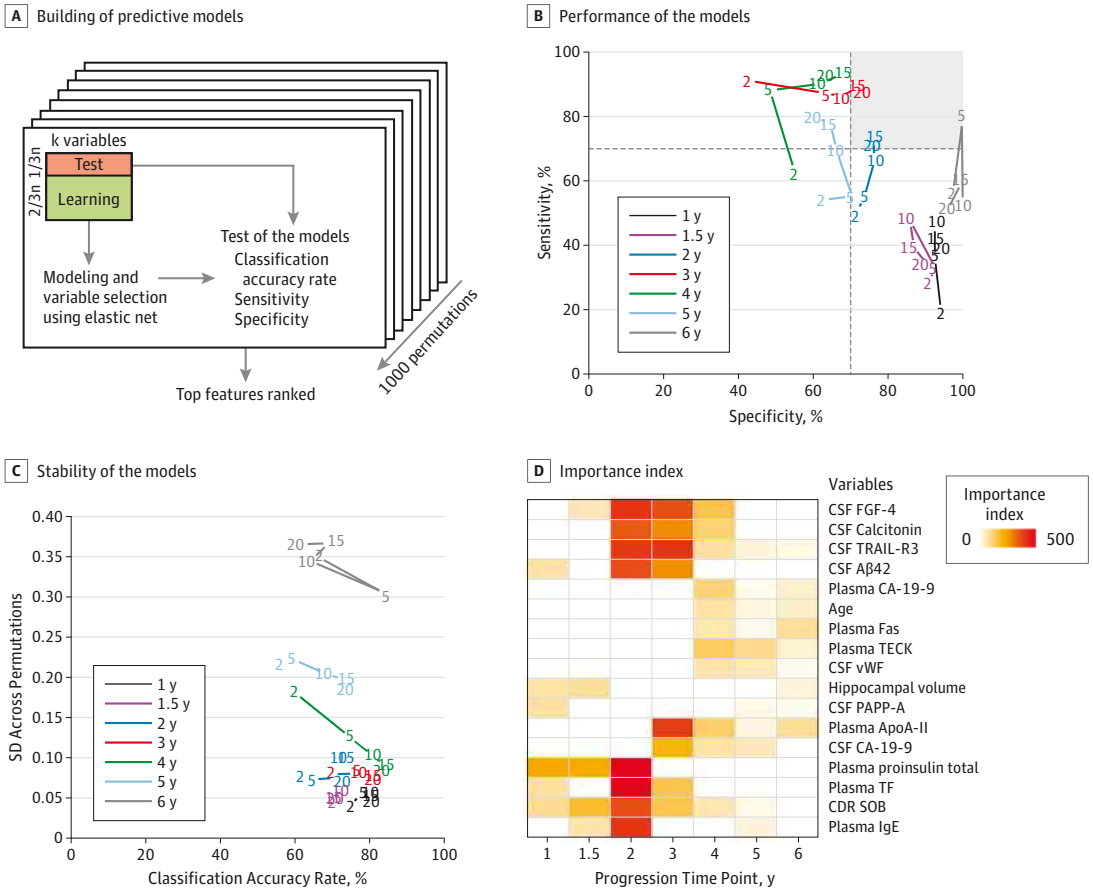
CSF fibroblast growth factor 4 (FGF-4) were included in 4 models, and 13 additional variables were selected at least 3 times, including CSF Aβ42, hippocampal volume, and age (the importance index for each variable is given in Figure 3D).

Importance of Specific Categories of Patient Variables to Prediction of Progression Within 3 Years

To investigate whether patient variables from specific categories are sufficient by themselves to predict progression to AD, we tested 7 models using only certain types of variables instead of variables from all groups as used above (**Figure 4**). We focused on progression to AD within 3 years because the best trade-off between sensitivity and specificity was obtained for this point; for clinical trial planning, this point is also the most relevant. Clinical and demographic characteristics and the number of *APOE4* alleles between patients with stable MCI and those with progression to AD within 3 years were similar (eTable 4 in the Supplement). Those with progression to AD had lower levels of CSF Aβ42 (P = .02, unpaired 2-tailed t test).

Classification accuracy and sensitivity (Figure 4 and eFigure 3A in the Supplement) were similarly high for all models except for those using AD CSF biomarkers and the *APOE4* genotype, MRI-based brain regional volumes, or cognitive scores. Models using the plasma and CSF communicomes or all variables were superior in accuracy and sensitivity to the other models. Specificity was relatively low for all models but again better for models using the plasma and CSF communicomes

Figure 3. Prediction of Progression From Mild Cognitive Impairment (MCI) to Alzheimer Disease (AD) Within 1 to 6 Years

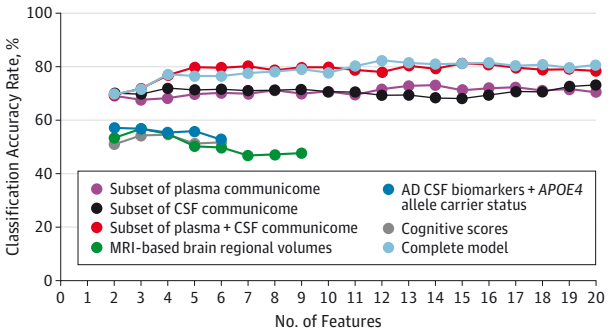


A, Strategy for modeling progression from MCI to AD uses an elastic net to perform joint modeling and variable selection. We performed 1000-fold resampling and ranked variables according to their number of appearances in the elastic net model across the 1000 permutations. The mean classification accuracy rate, sensitivity, and specificity were calculated across the 1000 resampled test data sets. K indicates the number of variables; n, sample size. B, Sensitivity as a function of the specificity for models includes the 2 to 20 top selected variables for each time point of progression. The number of variables are indicated by the numbers in the lines in the plot. Gray quadrant indicates the area of the figure in which sensitivity and specificity are greater than 0.7. C, Classification accuracy rate as a function of its SD across permutations for models includes the top 2 to 20 selected variables for each time point of progression. A small SD across permutations (close to 0) demonstrates a high stability of the model. D, Heatmap of variables consistently selected across time points of progression. A total of 80 of 224 variables were selected at least once as 1 of the top 20 variables in the different models. Details of the 80 variables are available in eTable 5 in the Supplement. Only variables selected in the top 20 of at least 3 progression time points are represented in the heatmap. The importance index represents the number of times a variable was selected across the 1000 permutations. Aβ indicates β-amyloid; ApoA-II, apolipoprotein A-II; CA-19-9, cancer antigen 19-9; CDR SOB, Clinical Dementia Rating Sum of Boxes composite test score; CSF, cerebrospinal fluid; FGF-4, fibroblast growth factor 4; PAPP-A, pregnancy-associated plasma protein A; TECK, thymus-expressed chemokine; TF, transferrin; TRAIL-R3, tumor necrosis factor–related apoptosis-inducing ligand receptor 3; and vWF, von Willebrand factor.

or all variables (eFigure 3B in the Supplement), making these 2 models the best overall.

Remarkably, only 6 plasma and CSF analytes were necessary to reach a maximal sensitivity of 88% and a specificity of 70% (eFigure 3A and B in the Supplement). The 6 analytes outperformed a model that included AD CSF biomarkers Aβ42, t-tau, and p-tau together with *APOE4* allele carrier status (Figure 4). Furthermore, this top model clearly performed better than randomly generated variables and is not improved by including the plasma Aβ42:Aβ40 ratio (eTable 6 in the Supplement). This signature was composed of 2 analytes measured in plasma (ApoA-II and cortisol) and 4 proteins measured in the CSF (FGF-4, heart-type fatty acid binding protein [FABP-heart], calcitonin, and TRAIL-R3). The mean level of TRAIL-R3 was significantly decreased for patients with progression to AD (mean, 0.63 [95% CI, 0.56-0.70] vs 0.81 [0.71-0.92]; $P < .01$, unpaired 2-tailed t test), whereas mean levels of ApoA-II (531 [478-583] vs 445 [402-487]), cortisol (165 [149-180] vs 141 [125-158]), and FGF-4 (49 [44-54] vs 39 [33-46]) were significantly increased (eFigure 4 in the Supplement; $P < .05$, unpaired 2-tailed t tests). Levels of calcitonin and FABP-heart were not significantly different between patients with stable MCI and those with progression to AD (t tests, $P = .07$ and $P = .14$, respectively). The levels of 4 of these 6 analytes in addition to CSF Aβ42 were significantly different between patients with stable MCI and progressive MCI, whereas CSF levels of t-tau and p-tau were not (eFigure 5 in the Supplement; $P = .28$ and $P = .70$, respectively). In summary, the levels of only 6 plasma

Figure 4. Prediction of Progression From Mild Cognitive Impairment to Alzheimer Disease (AD) Within 3 Years



Prediction includes 7 models combining different subsets of variables. Correct classification rate of the top 20 variables was estimated on the test data set after 1000-fold resampling of the learning and test data sets. Sex and age were included in all models. *APOE4* indicates apolipoprotein ε4; CSF, cerebrospinal fluid; and MRI, magnetic resonance imaging.

and CSF analytes were sufficient for the prediction of progression from MCI to AD within 3 years.

Discussion

In this study, we used data from the ADNI database and combined clinical and demographic data, cognitive measurements, brain volumetric data, *APOE* and translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) genotypes, and a large number of analyte measurements in the CSF and plasma to predict progression from MCI to AD during 6 years of patient follow-up. The ADNI database is a valuable resource to better understand AD clinically, particularly with respect to the integration of imaging data.[18] In contrast, comprehensive analyses with a broader range of clinical or biological variables, such as the one reported herein, are challenging owing to the rather patchwork collection of the data over time (Figure 1A). As exemplified herein, this approach results in a relatively small sample size for applicable patients with MCI (94 herein owing to the limited availability of the CSF multiplex data, for example), and identification of an appropriate independent validation data set is difficult or even impossible. These 2 points are probably the major limitations of our study. Despite these limitations, a network visualization approach allowed us to produce an overview of the complex relationships between and within categories of variables in ADNI (Figure 2). To overcome the shortcoming of missing data for modeling progression of MCI to AD, we performed thorough cross validation (eg, 1000 times resampling of the patients included in the learning and test data sets for an unbiased and robust estimate of the accuracy of the models[32]), evaluation of stability of the models to assess the potential generalization of findings from this study to other data sets,[33,34] and a final forward classification step to avoid overfitting of the predictive models. Altogether, the validation strategies applied by us are based on current standards in the field,[33-36] and we believe their combination resulted in the most rigorous validation that can be performed in the absence of an additional cohort.

A key question in modeling progression from MCI to AD relates to the temporal utility of measured variables. Of the features most consistently selected across progression time points (Figure 2D), 7 were analytes measured in CSF (including Aβ42) and 7 were analytes measured in plasma. Only 3 non-body fluid variables (CDR SOB, hippocampal volume, and age) were selected, and the *APOE4* genotype was not included despite the *APOE4* allele being the major genetic risk factor for AD.[37] The utility of *APOE4* allele status in predicting time to progression to AD is not clear because results have been inconsistent.[38-41] In contrast, age, which is the strongest environmental risk factor for developing sporadic AD,[42] was included in the model of long-term progression. A high rate of decline in hippocampal size is known to be one of the best MRI-based biomarkers of AD,[43,44] and hippocampal atrophy was selected as a predictor of short-term progression. The composite CDR SOB score was in the top features for almost all of the progressive time points studied (6 of 7). Our data indicate that the baseline CDR SOB composite test score combined with other variables could be useful for predicting short-term, midterm, and long-term progression from MCI to AD and support its use for planning and analyzing clinical trials.

Being able to select patients with midterm progression from MCI to AD is of major interest for assessing the efficacy of new AD therapies or for stratifying clinical trial cohorts. Herein, we demonstrated that signatures relying on prediction of progression within 2 and 3 years were more robust than those relying on other progressive time points in terms of sensitivity, specificity, and stability. At least for the time points after 4 years, this outcome may be influenced in large part by the lack of a sufficient sample size. Several other teams[32,38,41,45-48] analyzed the ADNI data and identified methods to predict progression from MCI to AD within 3 years, primarily focusing on imaging data. The best model so far had a classification accuracy rate close to what we report herein but a sensitivity of only 53%.[38] Although imaging is one of the best methods for monitoring AD, a blood test that predicts progression from MCI to AD within a defined period of time would be immensely useful because blood samples are easy to collect. However, other investigators[49] reported that using the ADNI data set plasma analytes alone could not adequately predict midterm progression to AD. Recently, 2 candidate signatures of progression to AD were proposed.[50,51] These studies were, however, limited to the prediction of progression to AD 1 year before its clinical diagnosis. In addition to plasma biomarkers, CSF-based biomarkers may be particularly representative of the disease progression because CSF is in close contact with the central nervous system. For instance, low CSF concentrations of Aβ42 in combination with high levels of t-tau and p-tau are sensitive and specific diagnostic biomarkers of AD.[17,52] In the entire population with MCI in the ADNI cohort, however, our study shows that CSF concentrations of these markers cannot be used to reliably predict time to progression to AD (Figure 1B). Consequently, inclusion of additional markers needs to be investigated, and we found that markers

in plasma or CSF used separately for modeling provided a relatively high sensitivity in detecting progression to AD (Figure 4). Once further validated, the marker sets in each of these fluids may be useful for patient enrichment in clinical trials, albeit perhaps with lower accuracy. Our data indicate that combining as few as 6 specific communicome markers measured in CSF and plasma may be more powerful in predicting the progression from MCI to AD and in identifying patients with stable MCI.

This finding is in line with what a group of investigators previously introduced as the communicome being "a reductionist approach to study brain aging and disease."[53(p185)] Because the plasma and CSF proteome is particularly challenging for unbiased proteomics approaches, such as mass spectrometry, measuring the secreted communication factors of cells is a straightforward way to explore the integrated response of cellular communication between tissues in physiological and pathophysiological states. Although this method is biased and restricted, it focuses on the proteome of key biological communication factors. Still, future studies should examine the diagnostic utility of the 6 markers to discriminate AD from other causes of dementia and to assess this signature and each candidate marker as a potential biomarker of cognitive decline in independent and larger sample sets. Indeed, individual communicome plasma and CSF factors—and thereby the proposed signature—can be influenced by variables such as age, sex, or ethnicity.

So far, independent evidence reported in other data sets supports an association of the top CSF and plasma markers identified in this study with AD. Of the 6 markers predicting progression to AD within 3 years, CSF levels of FABP-heart and TRAIL-R3 and plasma levels of cortisol and ApoA-II have already been reported by others to be involved in AD.[54-64] Levels of FABP-heart are increased in CSF samples from patients with progression of MCI to AD[54] and highly associated with t-tau and p-tau levels and the ratio of Aβ42 to tau.[55] Plasma cortisol levels reflect the degree of cognitive impairment in AD,[56] are associated with the presence of the *APOE4* allele,[57] and correlate with Aβ-plaque brain burden measured by Pittsburgh compound B–labeled PET.[58] High cortisol levels were also reported previously in plasma, serum, or CSF in patients with MCI and AD compared with controls[56,59,60] and are also associated with more rapidly increasing symptoms of dementia.[56,60] Apolipoproteins have been implicated in the cause of AD,[61-63] and low levels of plasma ApoA-II are associated with an increased risk for cognitive decline in cognitively normal individuals.[62] The TRAIL-R3 marker is involved in the regulation of apoptosis and upregulated in cognitively impaired individuals compared with controls.[64]

The apparent discrepancy between our finding for ApoA-II and TRAIL-R3 in patients with MCI and findings by others[62,64] in cognitively normal individuals can perhaps be explained by a possiblly different and so far unknown contribution of ApoA-II and TRAIL-R3 in the disease progression from normal cognition to MCI and from MCI to AD. We report herein an increase in ApoA-II levels in patients with progression of MCI to AD compared with stable MCI. This finding suggests that individual factors, such as ApoA-II levels, show a biphasic association with disease progression, an aspect that should be further explored in other data sets and biological experiments. Finally, to the best of our knowledge, no direct link among calcitonin, FGF-4, and AD or MCI has been reported previously. These proteins have multiple functions, including use in immune pathways that could link them to altered immune function in AD.[65]

Conclusions

We performed an integrative statistical analysis of the MCI data subset in the ADNI database and showed that the combination of selected plasma and CSF markers may be sufficient for the prediction of midterm progression from MCI to AD. We propose that such a large-scale analytical approach using the ADNI database could be applied to other similar large data sets in AD and beyond. Markers or signatures thereby identified could become helpful for early diagnosis and monitoring of patients, patient stratification in clinical trials, or personalizing existing or upcoming therapies.

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