Journal of Alzheimer's Disease xx (20xx) x–xx
DOI 10.3233/JAD-180905
IOS Press

Identification of a Simple and Novel Cut-Point Based Cerebrospinal Fluid and MRI Signature for Predicting Alzheimer's Disease Progression that Reinforces the 2018 NIA-AA Research Framework

Priya Devanarayan[a], Viswanath Devanarayan[b,c], Daniel A. Llano[d,e,*]
and for the Alzheimer's Disease Neuroimaging Initiative[1]
[a]*Souderton Area High School, Souderton, PA, USA*
[b]*Charles River Laboratories, Horsham, PA, USA*
[c]*Department of Mathematics, Statistics and Computer Science, University of Illinois at Chicago, IL, USA*
[d]*Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Champaign, IL, USA*
[e]*Carle Neuroscience Institute, Urbana, IL, USA*

Accepted 4 January 2019

Abstract. The 2018 NIA-AA research framework proposes a classification system with Amyloid-β deposition, pathologic Tau, and neurodegeneration (ATN) for diagnosis and staging of Alzheimer's disease (AD). Data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database can be utilized to identify diagnostic signatures for predicting AD progression, and to determine the utility of this NIA-AA research framework. Profiles of 320 peptides from baseline cerebrospinal fluid (CSF) samples of 287 normal, mild cognitive impairment (MCI), and AD subjects followed over a 3–10-year period were measured via multiple reaction monitoring mass spectrometry. CSF $A\beta_{42}$, total-Tau (tTau), phosphorylated-Tau (pTau-181), and hippocampal volume were also measured. From these candidate markers, optimal signatures with decision thresholds to separate AD and normal subjects were first identified via unbiased regression and tree-based algorithms. The best performing signature determined via cross-validation was then tested in an independent group of MCI subjects to predict future progression. This multivariate analysis yielded a simple diagnostic signature comprising CSF pTau-181 to $A\beta_{42}$ ratio, MRI hippocampal volume, and low CSF levels of a novel PTPRN peptide, with a decision threshold on each marker. When applied to a separate MCI group at baseline, subjects meeting these signature criteria experience 4.3-fold faster progression to AD compared to a 2.2-fold faster progression using only conventional markers. This novel 4-marker signature represents an advance over the current diagnostics based on widely used markers, and is easier to use in practice than recently published complex signatures. This signature also reinforces the ATN construct from the 2018 NIA-AA research framework.

Keywords: Biomarker, mild cognitive impairment, PTPRN, receptor-type tyrosine-phosphatase-like N

[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://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/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

*Correspondence to: Daniel Llano, MD, PhD, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Champaign, IL 61801, USA. E-mail: d-llano@illinois.edu.

INTRODUCTION

Tools to provide an early diagnosis and prediction of progression to Alzheimer's disease (AD) are of critical importance. Early diagnosis allows caregivers to plan for additional needs which will decrease the overall financial burden of the illness [1, 2]. In addition, early diagnosis may help identify common comorbidities such as depression or undernutrition [3, 4], and may spur lifestyle interventions to mitigate some of the cognitive impairments associated with aging [5]. Finally, identifying individuals who are more likely to progress will help enrich clinical trial populations with subjects with more rapid progression, potentially shortening trial duration.

Early pathological changes of AD are seen years before the clinical diagnosis of AD. Most studies have shown that individuals with mild cognitive impairment (MCI) carry AD pathological burden and have a substantial risk ($\sim$10–15% per year) of development of dementia [6, 7]. Thus, as new therapeutics are developed that target AD-related pathology, MCI may represent a state during which early intervention may change the trajectory of patient outcomes. However, therapeutics targeting Aβ will likely carry potential risks of significant side-effects, as documented in clinical trials [8–10], thus limiting their use to those with a high risk of subsequent cognitive decline. Therefore, what is needed is an approach to accurately identify MCI patients with the highest risk of conversion to AD.

Multiple potential biomarkers have been identified to aid in the prediction of conversion of MCI to AD. For example, cognitive and behavioral biomarkers have been proposed to identify individuals at high-risk for conversion [11–13]. In addition, biomarkers based on brain imaging or measurements in bodily fluids have been identified (for recent reviews, see [14, 15]). The latter groups of biomarkers have recently been organized into a generalizable research framework. This framework, labeled AT(N), describes three classes of biomarkers: 1) "A" or aggregated amyloid-based (e.g., cerebrospinal fluid (CSF) $A\beta_{42}$ levels, amyloid positron emission tomography (PET)), 2) "T" or aggregated tau-based (e.g., CSF phosphorylated tau [pTau-181], tau PET) and 3) "N" or neuronal degeneration-based (e.g., volumetric magnetic resonance imaging (MRI), fluorodeoxyglucose (FDG) PET, CSF total tau (tTau)) [16, 17]. Furthermore, Jack et al. [16] advocate extending this to the ATX(N) framework, where X can include additional markers from the multiarray–omics platforms. This research framework is intended to form a common approach by which investigators can communicate about and classify novel biomarkers, thereby allowing their more rapid integration into current research.

CSF-based biomarkers have been of interest since they represent an assessment of biochemical changes in the central nervous system. The most commonly-observed changes in the CSF of AD subjects have been a reduction of $A\beta_{42}$ and increase in pTau-181 [18, 19]. We recently identified a 16-analyte CSF signature which showed higher sensitivity and specificity than any combination of $A\beta_{42}$, tTau, and pTau-181 for the diagnosis of AD versus controls, and, when applied to an independent dataset of MCI subjects, outperformed traditional biomarkers in prediction of conversion to AD [20]. Unfortunately, a complicated 16-analyte signature is not practical for clinical purposes. Multi-analyte signatures require quality-control measures for each analyte and do not provide an intuitive understanding of how changes in the biomarker impact the disease. Therefore, in the current report, we modified the analytical approach to examine the ability of simpler signatures using intuitive cut-point thresholds to predict MCI to AD conversion. Such simplified signatures will be easier to implement in practice than a 16-peptide signature. We identify, using data from only the AD and age-matched Normal (NL) subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, optimal diagnostic signatures using novel CSF peptides combined with conventional CSF and volumetric MRI biomarkers to separate AD and NL subjects. Decision thresholds are determined on markers that separate AD and NL subjects via unbiased regression and tree-based algorithms [21, 22]. The best performing signatures from the NL and AD subjects were then tested in an independent group of MCI subjects at baseline to determine their ability to predict the future progression. The current approach of developing a biomarker signature on one group (AD versus NL) and testing on another (MCI progression or not) has successfully been applied previously [23] and avoids potential biases of approaches that split subjects into subgroups and develop a biomarker on one subgroup and evaluating on the other. The latter approach tends to produce signatures that are highly specific to the population under study and can inflate accuracy values. By developing a simple, yet powerful, cross-validated signature for prediction of MCI to AD, this work confirms and extends the AT(N) framework and provides

a potential new tool for clinicians to use to advise decision making and for researchers to enrich clinical trials with MCI subjects with a higher likelihood of conversion.

METHODS

Data used for this research were mostly identical to that used in Llano et al. [20], except that the progression data on MCI now extends to another two years, and we include data from the conventional biomarkers (CSF amyloid/tau and MRI hippocampal volume [HV]) in the analysis. For the sake of completeness, we repeat some of the key information pertaining to these data in this paper. The ADNI database (http://adni.loni.usc.edu) utilized in this research was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. For up-to-date information, see http://www.adni-info.org. This study was conducted across multiple clinical sites and was approved by the Institutional Review Boards of all of the participating institutions. Informed written consent was obtained from all participants at each site. Data used for the analyses presented here were accessed on February 24, 2018. Although the ADNI database continues to be updated on an ongoing basis, most newly added biomarker data are from later time points (i.e., beyond 1 year), in contrast to the baseline data used in this study.

Patient population

This research was focused on only those subjects in the ADNI database for whom data from both the conventional markers (CSF amyloid/tau and MRI HV) and novel markers (320 peptides from the multiple reaction monitoring (MRM) proteomics panel) were available at baseline. This included 287 subjects with AD, MCI, and NL from the ADNI study that received clinical, neuropsychological, and biomarker assessments which were repeated every six months for a period of 3 to 10 years. NL individuals were free of memory complaints or depression and had a Mini-Mental State Examination (MMSE) score above 25 and a Clinical Dementia Rating (CDR) score of 0. We note that 80/86 (93%) of NC subjects had an MMSE score of 28 or higher and that 2/86 (2.3%) had an MMSE score of 25 or 26. MCI individuals could have MMSE scores of 23 to 30 and required a CDR of 0.5 and an informant-verified memory complaint substantiated by abnormal education-adjusted scores on the Wechsler Memory Scale Revised—Logical Memory II. AD patients could have MMSE scores of 20 to 27 and a CDR of 0.5 or 1.0.

Imaging

All participants received 1.5 Tesla (T) structural MRI at baseline and at every six months for the next several years. In addition, approximately 25% also received 3.0 T MRI. Cognitive assessments and neuroimaging procedures were carried out within two weeks of each other. In this research, we utilized only the baseline HV data measured via MRI and computed using the FreeSurfer software at the University of California in San Francisco. Details regarding this software can be found in the "UCSF FreeSurfer Methods" PDF document under "MR Image Analysis" in the ADNI section of https://ida.loni.usc.edu/) as well as in [24–26].

CSF samples

CSF levels of $A\beta_{42}$, tTau, and pTau-181 were determined using Innogenetics' INNO-BIA AlzBio3 immunoassay on a Luminex xMAP platform (see [19] for details). These CSF samples were also processed in the Caprion Proteomics platform that uses mass spectrometry to evaluate the ability of a panel of peptides to discriminate between disease states and predict disease progression. The CSF multiplex MRM panel was developed by Caprion Proteomics in collaboration with the ADNI Biomarker Consortium Project Team. A total of 320 peptides generated from tryptic digests of 143 proteins were used in this study (see Supplementary Table 1 and the supplemental table in [20] for list of peptides and proteins).

Details regarding the technology, quality control and validation of the MRM platform can be found in the Use of Targeted Mass Spectrometry Proteomic Strategies to Identify CSF-Based Biomarkers in Alzheimer's Disease Data Primer (found under Biomarkers Consortium CSF Proteomics MRM Data Primer at http://ida.loni.usc.edu). In brief, as described in the data primer and in [27], plasma proteins were depleted from CSF samples using a Multiple Affinity Removal System (MARS-14) column, and digested with trypsin (1:25 protease:protein ratio). The samples were then

Table 1
Disease-state demographics

	AD ($n=66$)	MCI ($n=135$)	NL ($n=86$)
Gender (n)			
M	37	91	44
F	29	44	42
ApoE (n)			
E4	47	71	21
Non-E4	19	6	65
Age (years, Mean ± SD)	75.1 ± 7.5	74.8 ± 7.4	75.8 ± 5.6
Education (years, Mean ± SD)	15.1 ± 3	16 ± 3	15.6 ± 3
MMSE (Mean ± SD)	23.5 ± 1.9	26.9 ± 1.7	29.1 ± 1
	MCI to AD converters ($n=64$)	Stable MCI ($n=71$)	
Gender (n)			
M	40	51	
F	24	20	
ApoE (n)			
E4	40	31	
Non-E4	24	40	
Age (years, Mean ± SD)	74.9 ± 7.6	74.7 ± 7.2	
Education (years, Mean ± SD)	15.6 ± 3.0	16.4 ± 2.9	
MMSE (Mean ± SD)	26.4 ± 1.7	27.4 ± 1.6	

lyophilized, desalted and analyzed by LC/MRM-MS analysis on a QTRAP 5500 LC-MS/MS system at Caprion Proteomics. MRM experiments were performed on triple quadrupole (Q) mass spectrometers. The first (Q1) and third (Q3) mass analyzer were used to isolate a peptide ion and a corresponding fragment ion. The fragment ions were generated in Q2 by collision induced dissociation. All peptide levels are presented as normalized and log2-transformed intensities as we and others have done previously [20, 27], which is identical to the manner in which they were provided in the quality-controlled dataset.

Statistical methods

Before describing the details of the analysis steps, we first summarize the overall analysis process outlined in (Fig. 1). Raw expression data from the 320-peptide MRM panel was normalized as outlined in [27]. The optimal subset of features among the MRM panel plus the core CSF amyloid/tau markers and MRI hippocampal volume was first selected from the training set of AD and NL subjects [28, 29]. Then signatures in the form of simple decision thresholds on each marker were derived by applying two multivariate subgroup identification algorithms [21, 22] on this training set. The performance of these signatures for differentiating the AD and NL subjects were then assessed via an internal cross-validation procedure, within which the feature selection and signature derivation process were fully embedded [30]. The optimally performing signature was then selected from this cross-validation procedure on the training set. This optimal signature was then applied on a separate group of MCI subjects at baseline to determine its ability to predict the future progression of these MCI subjects to AD. We now provide more details on the different steps of this analysis process.

Most algorithms in the predictive modeling and machine learning literature yield signatures in the form of a mathematical equation (e.g., logistic regression) or a large multi-layer decision tree (e.g., classification tree models) or a complex model without an explicit closed-form mathematical equation (e.g., random forests, support vector machines). Therefore, most multivariate biomarker signatures that have been proposed in the AD literature take such complex forms and are often based on numerous markers (e.g., 16-marker signature in [20] and 29-marker signature in [27], and is part of the reason for their lack of translation and use in the clinic.

One of the important analysis objectives in this paper was to derive biomarker signatures that are more amenable for routine clinical use and practice. Therefore, we wanted the signatures to be as small as possible (i.e., as few markers as possible), and to take a simple form of a binary decision rule that is enabled by a threshold (cut point) on each marker. We call

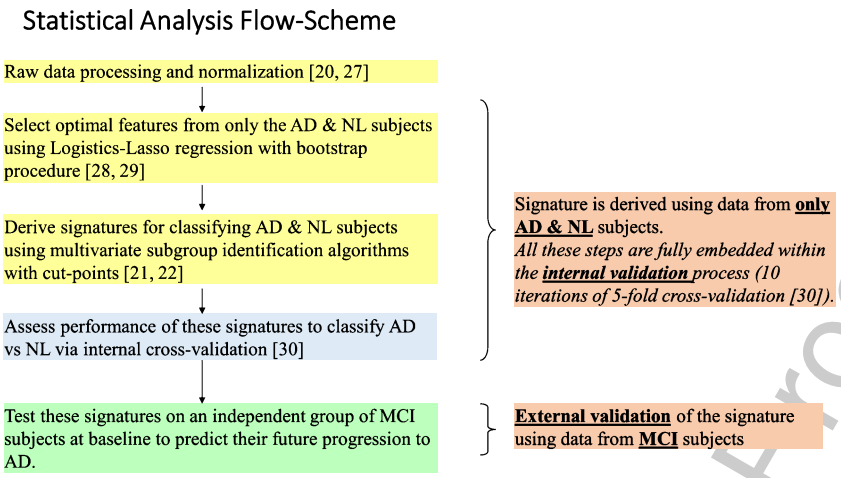

Fig. 1. Statistical analysis flow-scheme.

such signatures as multivariate threshold-based signatures. When applying such a signature in practice, one would simply compare the measure level of each marker from this signature to its decision threshold in order to predict the phenotype of the subject (e.g., AD or NL diagnosis, or whether not an MCI subject at baseline will progress to AD in the future), without the need for complex calculations, formulae or a mathematical model.

As noted above, there are relatively fewer algorithms in the statistical literature for deriving such multivariate threshold-based signatures. We use two recently published nonparametric subgroup-identification algorithms called Patient Rule Induction Method (PRIM) [21] and Sequential Bootstrapping and Aggregation of Trees (BATTing) [22]. These algorithms were found to significantly outperform the current benchmark algorithms in the predictive modeling literature [22]. The PRIM algorithm identifies regions in the training dataset where patients present a target phenotype such as disease diagnosis or progression in our case. This is accomplished via an iterative combination of peeling and pasting steps with respect to optimal markers, where small fractions of the data are removed or added to the current region, until the region is optimized for the target phenotype. The sequential BATTing algorithm sequentially stratifies the training dataset with respect to the most optimal markers one at a time, with the optimal thresholds on each marker estimated via a resampling approach. Further details on these algorithms are provided in [21, 22].

We employ both these algorithms in our analysis, and the best performing signatures are chosen via the internal cross-validation procedure within the AD versus NL training set. Prior to the application of these algorithms on the amyloid/tau markers, MRI hippocampal volume, and 320-peptide MRM panel, an optimal subset of promising markers (features) were first selected within the training set of AD and NL subjects via a Lasso-based regularization method [28], along with a bootstrap resampling procedure [29] to improve the stability of the feature selection process. This process does not require the specification of a certain number of features because the optimal number is selected based on maximizing the differentiation of AD and NL subjects within this training set [28, 29]. The application of PRIM and Sequential BATTing algorithms on this optimal subset of features then yields signatures in the form of simple decision thresholds on each of the optimal features [22]. As explained earlier in this section and as illustrated in Fig. 1, the feature selection and signature derivation process are fully embedded within the rigorous internal cross-validation framework that is explained below.

The predictive performance of the optimal signature from each algorithm for differentiating the AD and NL subjects within the training set was evaluated via 10 iterations of five-fold internal cross-validation. In this procedure, the training set data were first divided into five random subsets (folds). Each fold was left out one at a time, and the remaining four folds were used to derive a signature. This signature was then used to predict the AD or NL disease state of each subject in the left-out fold. This process was carried out for each left-out fold one at a time, and the predictions of all the five left-out folds were then

aggregated. Performance measures such as the positive and negative predictive value (PPV, NPV) and overall accuracy were calculated by comparing the predicted AD versus NL status of each subject in the left-out fold to the true diagnostic status. For better reliability and robustness of these performance measures, this internal cross-validation procedure was repeated 10 times and the median of each these performance measures was calculated. Most importantly, all steps of the signature derivation process, including the feature selection process mentioned above, were fully embedded within this cross-validation to further reduce any possible bias [31]. This method for robust estimation of the performance of multivariate signatures in predictive modeling has been recommended in the white-paper by bioinformatics experts from the FDA, industry and academic in the Microarray Quality Control Consortia working group [30].

The optimal signature from the best performing algorithm (i.e., the signature that best differentiated AD and NL subjects in the internal cross-validation) was now tested on a separate independent group of 135 MCI subjects at baseline, to predict their future progression to AD. As this signature takes the form of a simple decision rule with a cut point on each marker in the signature, no mathematical equation or model fitting was needed for the prediction of each subject. The MCI subjects whose markers at baseline satisfied the cut points of this signature were predicted to be AD-like (called "Signature Positive") and therefore considered as future converters to AD. The MCI subjects whose markers at baseline did not satisfy the cut points of this signature were predicted to be NL-like (called "Signature Negative") and therefore considered as non-converters.

These baseline predictions of the MCI subjects were then compared to the follow-up clinical data. Performance metrics such as the PPV, NPV, and overall accuracy were calculated by comparing the predictions to the known progression status of the MCI subjects to AD over the next 36 months. Comparisons of the performance metrics between different signatures were carried out via exact McNemar's test.

These multivariate threshold-based signatures were then evaluated for ability to differentiate the future time to progression of the MCI subjects to AD. This was accomplished by comparing the time for MCI to AD progression of the predicted signature positive MCI subjects at baseline (i.e., the MCI subjects that satisfied the cut points of the markers this signature at baseline) versus the predicted signature-negative MCI subjects at baseline via Kaplan-Meier analysis. For this evaluation, the progression of MCI subjects to AD over the entire future time course until the last follow-up visit (up to 120 months) was taken into consideration.

This analysis procedure was carried out separately for the following subsets of markers, along with APOE genetic status, age, gender and education (4 markers):

- MRI brain HV: 5 total markers (the 4 markers above + HV)
- CSF $A\beta_{42}$, tTau, pTau-181, ratios of tTau to $A\beta_{42}$ & pTau-181 to $A\beta_{42}$ (AT): 9 total markers
- AT + HV: 10 total markers, and
- AT + HV + 320 peptides from the CSF MRM panel: 330 total markers.

While it is not necessary for a signature that differentiates AD versus NL subjects to predict the progression of MCI subjects to AD, we wanted a signature that predicts disease progression to also be relevant for disease diagnosis as it would better reflect the AD pathology. Most importantly, this evaluation of the AD versus NL signature on the MCI subjects at baseline to predict their future progression to AD not only served as an independent verification of the utility of our signature, but also put it to a much greater test to see whether it is robust enough to address a different and more important question related to the prediction of future progression of the MCI subjects to AD. The analysis procedure described here is summarized in (Fig. 1).

The outliers shown in the box-plot figures meet the following criteria; samples results greater than Q3 + 1.5 × (Q3–Q1) or lower than Q1–1.5 × (Q3–Q1), where Q1 and Q3 are the 25th and 75th percentiles respectively. Although these outliers are shown in the figures, they were not excluded from the analysis to derive optimal signatures. This is because the algorithms used in our analysis (Sequential BATTing and PRIM; [21, 22]) are nonparametric (distribution-free) and robust to extreme values.

All analyses related to predictive modeling and signature derivation were carried out using R (http://www.R-project.org), version 3.4.1, with the publicly available package, SubgrpID [22]. The time to progression analysis of the derived signatures and related assessments were carried out using JMP®, version 13.2.

RESULTS

Disease-state demographics

Table 1A summarizes the key demographics of the 66 AD, 135 MCI, and 86 NL subjects, and Table 1B provides a breakdown of the 135 MCI subjects in terms of their future progression. The subjects were balanced across groups in terms of age and education (both $p > 0.05$). There were significantly more males (59.1%) than females (40.9%) in the study, though similar numbers of male and female MCI subjects converted to AD over a three-year period (44% versus 54.6%, $p = 0.248$, Chi-squared test). As shown previously [32], the presence of at least one copy of the APO-E4 allele was a risk factor for AD (71.2% AD, 52.6% MCI and 24.4% NL, $p < 0.0001$, Chi-squared test). In addition, this allele also tracked with MCI to AD progression over a 36-month period (37.5% of non-E4 versus 56.3% of E4 progressed to AD, $p = 0.029$, Chi-squared test).

Disease state classification

The distribution of the conventional biomarkers for NL, MCI, and AD subjects are shown in (Fig. 2A-D). While the means significantly differ across groups ($p < 0.0001$), the considerable overlap of expression levels greatly limits the diagnostic utility of any of these markers on their own.

Multivariate analysis of the various markers using data-driven computational algorithms described above yielded optimal signatures for differentiating the disease states and prediction of disease progression. These signatures are summarized in (Table 2). Interestingly, the signature derived from the conventional and novel markers took a very simple form based on only a few markers, with representations from both the conventional markers and the novel MRM panel, along with a cut-point on each of them; it took the form of HV $<7.65\,cm^3$, ratio of pTau to $A\beta_{42}$ >0.09 and a PTPRN peptide (sequence SELEAQTGLQILQTGVGQR, referred to here as PTPRN.SELE) <10.22 intensity units. Figure 2E and F show the significant decline of PTPRN.SELE in AD relative to both NL ($p = 0.002$) and MCI ($p = 0.004$), and a trend toward a decline in baseline MCI subjects that progress to AD in the future ($p = 0.065$).

Prediction of MCI to AD progression

For disease state classification, the signatures derived from all data scenarios have similar levels of overall accuracy, with no discernable advantage of adding novel markers from the MRM panel to the conventional markers. However, for the prediction of 36-month progression in the independent group of 135 MCI subjects at baseline, the signature derived from the collection of both conventional and novel markers significantly outperforms the signatures based on the conventional markers ($p = 0.0002$), with the NPV increasing from 70.2% to 77.6% ($p = 0.0032$) and the PPV increasing slightly from 60.2% to 61.6% ($p = 0.0107$). Thus, the addition of a novel PTPRN peptide from the MRM panel to the conventional AD markers substantially improves the prediction of 36-month disease progression in MCI subjects at baseline.

Based on the available 3–10-year follow-up clinical data available on these subjects, the performance of the optimal signatures from all the scenarios was further assessed on this independent group of baseline MCI subjects with respect to their future time to progression. Table 3 includes a summary of the 25th percentile, median, and 75th percentile time to progression of the signature negative and signature positive subjects, and the overall hazard ratio with 95% confidence bands. Based on these results, the optimal combination of conventional markers showed a hazard ratio of 2.2 suggesting that the MCI subjects meeting the criteria of this signature experience 2.2-fold faster progression to AD. However, the MCI subjects that meet the signature criterion from the scenario that includes the PTPRN peptide experience 4.3-fold faster progression to AD, as shown in (Fig. 3). To determine if the impact of PTPRN was likely isolated to the particular peptide fragment (PPRN.SELE, the other two PTPRN peptides, AEAPALFSR, referred to as PTPRN.AEAP and LAAVLAGYGVELR, referred to as PTPRN.LAAV) in the MRM panel were also assessed. The pairwise correlations between these three peptides are all over 87% (data not shown).

There were more male ($n = 91$) MCI subjects than female ($n = 44$) and, as described above, similar proportions (40/91 and 24/44, $p = 0.248$, Chi-squared test) converted to AD over 36 months. Among the female subjects, the optimal signature had an NPV of 87.5% and a PPV of 63.9%. Among the

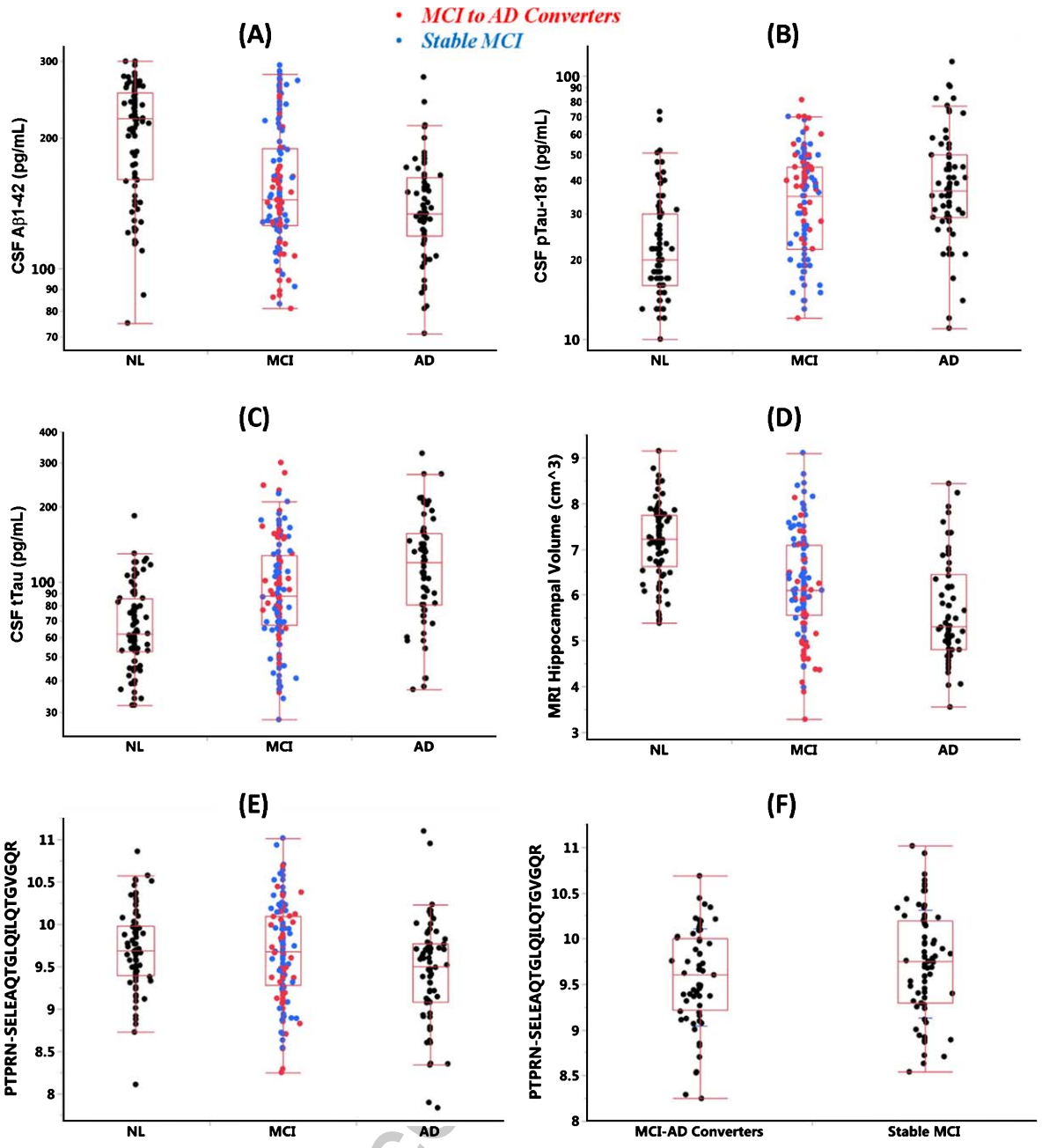

Fig. 2. Distribution of four conventional markers of AD (A: CSF $A\beta_{42}$, B: CSF pTau-181, C: CSF tTau, D: MRI HV) are shown for the NL, MCI and AD subjects at baseline. Among the MCI subjects, those that progressed to AD over 36 months are shown in red and rest are shown in blue. The bottom and top ends of the box represent the first and third quartiles respectively, with the line inside the box representing the median. Lines extending out of the ends of the box indicate the range of the data, minus the outliers. The points outside the lines are the low and high outliers. E) Distribution of PTPRN.SELE peptide (in normalized log2 transformed intensity units) is shown across the NL, MCI and AD groups at baseline, and F) for the baseline MCI subjects that either progressed to AD or remained stable over the next 36 months.

male subjects these numbers were 75.6% and 60%, respectively. Given the small numbers of female signature-negative subjects ($n = 8$), no statistical comparison was made for NPV and PPV values between gender groups. However, the values are qualitatively similar, suggesting that there is no significant impact of gender on the utility of optimal signature to predict MCI to AD conversion.

Table 2
Performance of optimal signatures

Data type	Diagnostic Criteria for Signature positive	AD versus Normal Diagnosis (internal cross-validation)			36 m MCI Progression to AD (independent validation)		
		PPV	NPV	Accuracy	PPV (MCI to AD)	NPV (Stable MCI)	Accuracy
AT	tTau/A$\beta_{1\text{-}42}$ >0.59	71.6%	80.5%	76.5%	58.1%	66.1%	61.7%
HV	HV <6.41 and ApoE4+	92.7%	74.8%	79.6%	61.2%	60.5%	60.7%
AT + HV	HV <7.0, pTau >18.1, and tTau/A$\beta_{1\text{-}42}$ >0.36	73.4%	78.4%	76.3%	60.2%	70.2%	64.4%
AT + HV +	HV <7.65, pTau/A$\beta_{1\text{-}42}$ >0.09, and PTPRN.SELE < 10.22	75%	79.6%	77.6%	61.6%	77.6%	67.4%

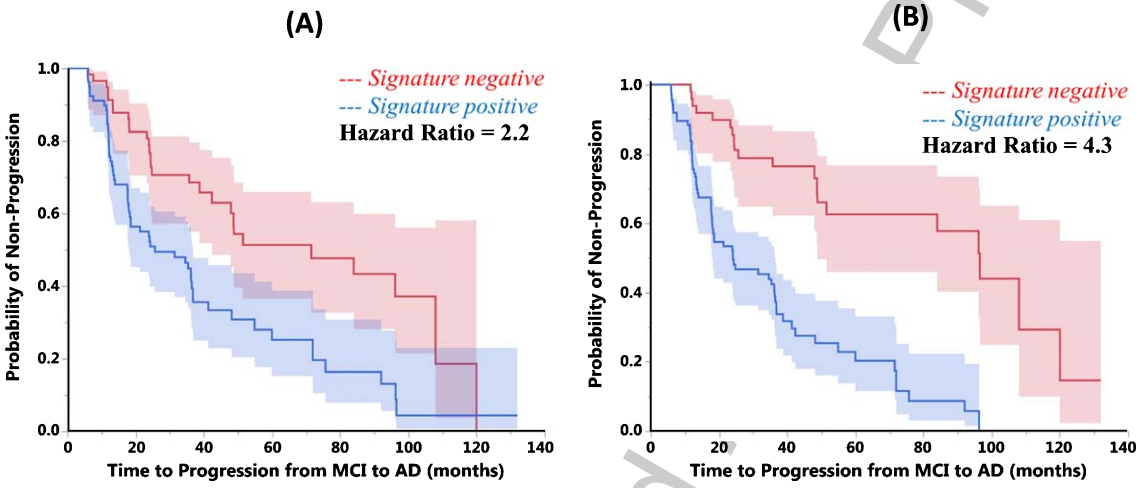

Fig. 3. Time to progression profiles of the signature positive versus signature negative MCI subjects with the shaded 95% confidence bands are shown here via Kaplan-Meier analysis. The effect of signature based on only the conventional markers (HV and AT) is illustrated in (A) and the signature with both the conventional markers and the novel PTPRN.SELE peptide from the MRM panel is shown in (B). Patients meeting the signature criterion that includes this PTPRN peptide experience 4.3-fold faster progression to AD (hazard ratio = 4.4), relative to the 2.2-fold faster progression without this peptide.

Table 3
Time to progression (T2P) of MCI subjects to AD using optimal signatures

Data type	Diagnostic Criteria for Signature positive	Signature Negative		Signature Positive		Hazard Ratio (95% C.I.)
		N	T2P (months) Q1, Q2, Q3	N	T2P (months) Q1, Q2, Q3	
AT	tTau/A$\beta_{1\text{-}42}$ >0.59	59	23.4, 71.6, 108	76	13.6, 25.7, 72.0	1.9 (1.2, 3.1)
HV	HV <6.41 and ApoE4+	86	18.6, 48.2, 108	49	13.1, 31.5, 60.0	2.0 (1.3, 3.2)
AT + HV	HV <7.0, pTau >18.1, and tTau/A$\beta_{1\text{-}42}$ >0.36	57	24.4, 71.6, 108	78	12.6, 25.7, 72.0	2.2 (1.4, 3.6)
AT + HV + MRM	HV <7.65, pTau/A$\beta_{1\text{-}42}$ >0.09, and PTPRN.SELE <10.22	49	48.0, 96.5, 120	86	12.7, 24.1, 54.9	4.3 (2.5, 7.7)

Other peptides and prediction of MCI to AD conversion

In the current study, PTPRN emerged via an unbiased algorithm as the optimal analyte to combine with conventional biomarkers for disease-state classification. It should be noted that other CSF peptides have previously been shown to enhance prediction of MCI to AD conversion [20, 27, 33–37]. We therefore examined the performance of other peptides by sequentially removing the top-performing peptide from the MRM pool and re-running the unbiased algorithm to identify a new signature for disease-state classification. This signature was then used to predict

Table 4
Time to progression (T2P) of MCI subjects to AD using optimal and other candidate signatures for the AT+HV+MRM scenario. Please see text for definitions of abbreviations

Diagnostic Criteria for Signature positive	Signature Negative		Signature Positive		Hazard Ratio (95% C.I.)
	N	T2P (months) Q1, Q2, Q3	N	T2P (months) Q1, Q2, Q3	
HV <7.65, pTau/Aβ_{1-42} >0.09, and ***PTPRN.SELE*** <10.22	49	48.0, 96.5, 120	86	12.7, 24.1, 54.9	**4.3** (2.5, 7.7)
HV <7.99, pTau/Aβ_{1-42} >0.07, tTau /Aβ_{1-42} >0.25, ***FABPH.SIVT*** > 13.96, and ***NPTXR.ELDV*** <22.44	42	38.8, 96.6, 120	93	13.3, 24.3, 71.6	**3.8** (2.2, 7.1)
HV <7.61, tTau/Aβ_{1-42} >0.28, pTau >16.65, tTau >58, and ***AMD.IVQF*** <21.95	54	34.6, 84.0, 120	81	12.7, 24.1, 54.9	**3.3** (2.0, 5.5)

MCI-AD conversion. The top three resulting signatures and their performances are shown in Table 4. As shown, several other peptides improve predictive performance beyond conventional biomarkers alone. For example, combinations of heart fatty-acid binding protein (FABPH.SIVT) with the neuronal pentraxin receptor (NPTXR.ELDV) as well as peptidyl-glycine alpha-amidatingmonooxygenase (AMD.IVQF) have hazard ratios of 3.8 and 3.3 respectively, outperforming conventional biomarkers (HV+AT) which have a hazard ratio of 2.2 (Table 3).

DISCUSSION

Summary

We examined the ability of a simple optimized multivariate signature comprising conventional biomarkers combined with an array of novel CSF peptides from the ADNI database to both classify AD disease state and to predict MCI to AD conversion. We observed that both conventional AD biomarkers (HV and CSF pTau/Aβ_{42} ratio) and conventional biomarkers combined with an array of novel CSF peptides performed similarly in terms of classifying disease state (AD versus NL). However, when these optimized signatures were applied to an independent group of MCI subjects, the signature combining conventional markers with a novel peptide analyte derived from PTPRN substantially outperformed the conventional biomarkers in predicting MCI to AD conversion by nearly twofold. In addition, the combined signature contains only four elements: HV, CSF Aβ_{42}, tTau, and the PTPRN.SELE peptide, thus making it simple enough to be tractable for clinical and research purposes. These data may also open new lines of investigation regarding the role of PTPRN in AD as well as confirming and extending the proposed AT(N) framework for AD biomarkers.

PTPRN and AD

PTPRN is expressed widely in neurons throughout the mouse and human brain, including areas associated with AD neurodegeneration such as hippocampus and neocortex [38, 39]. It is a membrane-spanning protein phosphatase with cytoplasmic and luminal components and is found in the membranes of secretory granules. The gene for PTPRN is also highly expressed in pancreatic islet cells, and antibodies against this protein are found in type 1 diabetes, hence its alternative name islet-antigen 2 [40]. Deficiency in PTPRN is associated with glucose intolerance in animal models [41] as well as impaired learning [42]. Given the associations between diabetes, insulin resistance and AD [43–45], it is possible that the PTPRN/AD association seen in the current study points to a new and specific role for metabolic dysregulation in the pathophysiology of AD to complement other metabolic hypotheses of AD [46, 47].

Several previous studies have identified PTPRN as a potential marker of AD. For example, downregulation of the expression of the PTPRN gene has been observed in the hippocampus of sporadic AD subjects [48] as well as the posterior cingulate area of early-onset AD and presenilin-1 mutation-related dementia [49]. In addition, when incorporated into a three-gene classifier, PTPRN expression levels have been found to discriminate between patients with AD pathology and no symptoms, and those with only AD pathology [50]. Finally, in a preliminary study of genetic interactions with CSF pTau levels for predicting MCI to AD conversion, PTPRN levels showed differences with

respect to CSF pTau levels in MCI to AD converters compared to non-converters [51].

It is important to note that other CSF peptides may also be used in conjunction with conventional biomarkers to predict MCI to AD conversion. Here, we found that heart fatty-acid binding protein, the neuronal pentraxin receptor, as well as peptidyl-glycine alpha amidatingmonooxygenase, when combined with conventional biomarkers, all predict MCI to AD conversion better than conventional biomarkers alone (Table 4). While all of these peptides have previously been implicated in neurodegenerative disease [34, 35, 52–60], they do not outperform the combined PTPRN+AT+HV signature, which has the additional advantage of containing only four markers, thus amenable to use in clinical practice.

Implications of the prediction of MCI-AD conversion

Over the years, several groups have examined the ability of multi-modal combination biomarkers (i.e., combinations of imaging, cognitive, body fluid, and other markers) to predict the conversion of MCI to AD. Ideally, utilizing an approach such as the AT(N) framework, a combination biomarker should merge several orthogonal measurements reflecting different underlying biological processes. Larger combinations of biomarkers have the potential to increase the predictive power of the combination biomarker. The multiplicity of biomarkers is limited by clinical reality such that it is often impractical and costly to obtain multiple studies in individual patients. Therefore, a challenge in developing combination biomarkers is to develop combinations that provide high predictive MCI to AD accuracy and are clinically feasible.

Here, we have identified a 4-marker signature that combines volumetric MRI and CSF testing, both feasible clinical tests, that outperforms standard biomarkers in the prediction of MCI to AD. Although other studies have found that combinations of volumetric MRI and CSF measures can predict MCI to AD conversion [61–66], a unique aspect of the current biomarker signature is that it was initially developed using disease state markers from one population of subjects, and then validated on an independent group of individuals with MCI, increasing its generalizability. As expected, the external cross-validation used in the current study diminished the accuracy values compared to those observed for internal validation (see Table 2). However, the accuracy of MCI to AD prediction of current "gold standard" biomarkers of AT and HV was substantially bolstered by adding a single additional peptide (PTPRN). In addition, because the 4-marker signature is in the form of simple decision cut-points, it can readily be applied for clinical trial patient enrollment and in clinical practice for physicians without the need for complex calculations. It will be beneficial in the future to evaluate the performance of this signature in databases containing other neurodegenerative diseases to determine the specificity of these markers against related illnesses. In addition, further evaluation and validation of PTPRN as a diagnostic and progression marker for patients with early signs of cognitive impairment, in conjunction with the core beta-amyloid and tau markers, in line with the ATN construct proposed in the 2018 NIA-AA consensus paper may provide additional insights about AD pathology.

ACKNOWLEDGMENTS

The authors thank Dr. Clifford Jack from the Mayo Clinic for his constructive comments about an earlier version of this manuscript. The authors also thank Dr. Danielle Harvey from UC Davis for providing valuable input regarding the ADNI imaging data.

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.;Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health

Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (http://www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/18-0905r2).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-180905.

REFERENCES

[1] Leifer BP (2003) Early diagnosis of Alzheimer's disease: Clinical and economic benefits. *J Am Geriatr Soc* **51**, S281-S288.

[2] Prince M, Bryce R, Ferri C (2011) *World Alzheimer Report 2011: The benefits of early diagnosis and intervention*, Alzheimer's Disease International, London, UK.

[3] Steinberg M, Shao H, Zandi P, Lyketsos CG, Welsh-Bohmer KA, Norton MC, Breitner JC, Steffens DC, Tschanz JT (2008) Point and 5-year period prevalence of neuropsychiatric symptoms in dementia: The Cache County Study. *Int J Geriatr Psychiatry* **23**, 170-177.

[4] Roque M, Salva A, Vellas B (2013) Malnutrition in community-dwelling adults with dementia (NutriAlz Trial). *J Nutr Health Aging* **17**, 295-299.

[5] Ngandu T, Lehtisalo J, Solomon A, Levälahti E, Ahtiluoto S, Antikainen R, Bäckman L, Hänninen T, Jula A, Laatikainen T (2015) A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): A randomised controlled trial. *Lancet* **385**, 2255-2263.

[6] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999) Mild cognitive impairment: Clinical characterization and outcome. *Arch Neurol* **56**, 303-308.

[7] Small GW, Kepe V, Ercoli LM, Siddarth P, Bookheimer SY, Miller KJ, Lavretsky H, Burggren AC, Cole GM, Vinters HV (2006) PET of brain amyloid and tau in mild cognitive impairment. *N Engl J Med* **355**, 2652-2663.

[8] Egan MF, Kost J, Tariot PN, Aisen PS, Cummings JL, Vellas B, Sur C, Mukai Y, Voss T, Furtek C (2018) Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. *N Engl J Med* **378**, 1691-1703.

[9] Ketter N, Brashear HR, Bogert J, Di J, Miaux Y, Gass A, Purcell DD, Barkhof F, Arrighi HM (2017) Central review of amyloid-related imaging abnormalities in two phase III clinical trials of bapineuzumab in mild-to-moderate Alzheimer's disease patients. *J Alzheimers Dis* **57**, 557-573.

[10] Bayer AJ, Bullock R, Jones RW, Wilkinson D, Paterson K, Jenkins L, Millais S, Donoghue S (2005) Evaluation of the safety and immunogenicity of synthetic Aβ42 (AN1792) in patients with AD. *Neurology* **64**, 94-101.

[11] Llano DA, Laforet G, Devanarayan V (2011) Derivation of a new ADAS-cog composite using tree-based multivariate analysis: Prediction of conversion from mild cognitive impairment to Alzheimer disease. *Alzheimer Dis Assoc Disord* **25**, 73-84.

[12] Swords GM, Nguyen LT, Mudar RA, Llano DA (2018) Auditory system dysfunction in Alzheimer disease and its prodromal states: A review. *Ageing Res Rev* **44**, 49-59.

[13] Anderson ED, Wahoske M, Huber M, Norton D, Li Z, Koscik RL, Umucu E, Johnson SC, Jones J, Asthana S (2016) Cognitive variability—A marker for incident MCI and AD: An analysis for the Alzheimer's Disease Neuroimaging Initiative. *Alzheimers Dement (Amst)* **4**, 47-55.

[14] Henriques AD, Benedet AL, Camargos EF, Rosa-Neto P, Nóbrega OT (2018) Fluid and imaging biomarkers for Alzheimer's disease: Where we stand and where to head to. *Exp Gerontol* **107**, 169-177.

[15] Blennow K, Dubois B, Fagan AM, Lewczuk P, de Leon MJ, Hampel H (2015) Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. *Alzheimers Dement* **11**, 58-69.

[16] Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**, 535-562.

[17] Jack CR, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS (2016) A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* **87**, 539-547.

[18] Sunderland T, Linker G, Mirza N, Putnam KT, Friedman DL, Kimmel LH, Bergeson J, Manetti GJ, Zimmermann M, Tang B, Bartko JJ, Cohen RM (2003) Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. *JAMA* **289**, 2094-2103.

[19] Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H, Simon A, Lewczuk P (2009) Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* **65**, 403-413.

[20] Llano DA, Bundela S, Mudar RA, Devanarayan V, Alzheimer's Disease Neuroimaging Initiative (2017) A multivariate predictive modeling approach reveals a novel CSF peptide signature for both Alzheimer's disease state classification and for predicting future disease progression. *PLoS One* **12**, e0182098.

[21] Chen G, Zhong H, Belousov A, Devanarayan V (2015) A PRIM approach to predictive-signature development for patient stratification. *Stat Med* **34**, 317-342.

[22] Huang X, Sun Y, Trow P, Chatterjee S, Chakravartty A, Tian L, Devanarayan V (2017) Patient subgroup identification for clinical drug development. *Stat Med* **36**, 1414-1428.

[23] Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, Herukka S-K, van der Flier WM, Blankenstein MA, Ewers M (2009) CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* **302**, 385-393.

[24] Dale AM, Fischl B, Sereno MI (1999) Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage* **9**, 179-194.

[25] Fischl B, Sereno MI, Dale AM (1999) Cortical surface-based analysis: II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage* **9**, 195-207.

[26] Fischl B, Sereno MI, Tootell RB, Dale AM (1999) High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum Brain Mapp* **8**, 272-284.

[27] Spellman DS, Wildsmith KR, Honigberg LA, Tuefferd M, Baker D, Raghavan N, Nairn AC, Croteau P, Schirm M, Allard R, Lamontagne J, Chelsky D, Hoffmann S, Potter WZ; Alzheimer's Disease Neuroimaging Initiative; Foundation for NIH (FNIH) Biomarkers Consortium CSF Proteomics Project Team (2015) Development and evaluation of a multiplexed mass spectrometry based assay for measuring candidate peptide biomarkers in Alzheimer's Disease Neuroimaging Initiative (ADNI) CSF. *Proteomics Clin Appl* **9**, 715-731.

[28] Friedman J, Hastie T, Tibshirani R (2010) Regularization paths for generalized linear models via coordinate descent. *J Stat Softw* **33**, 1.

[29] Efron B, Tibshirani RJ (1994) *An introduction to the bootstrap*, CRC Press.

[30] Shi L, Campbell G, Jones WD, Campagne F, Wen Z, Walker SJ, Su Z, Chu TM, Goodsaid FM, Pusztai L, Shaughnessy JD Jr, Oberthuer A, Thomas RS, Paules RS, Fielden M, Barlogie B, Chen W, Du P, Fischer M, Furlanello C, Gallas BD, Ge X, Megherbi DB, Symmans WF, Wang MD, Zhang J, Bitter H, Brors B, Bushel PR, Bylesjo M, Chen M, Cheng J, Cheng J, Chou J, Davison TS, Delorenzi M, Deng Y, Devanarayan V, Dix DJ, Dopazo J, Dorff KC, Elloumi F, Fan J, Fan S, Fan X, Fang H, Gonzaludo N, Hess KR, Hong H, Huan J, Irizarry RA, Judson R, Juraeva D, Lababidi S, Lambert CG, Li L, Li Y, Li Z, Lin SM, Liu G, Lobenhofer EK, Luo J, Luo W, McCall MN, Nikolsky Y, Pennello GA, Perkins RG, Philip R, Popovici V, Price ND, Qian F, Scherer A, Shi T, Shi W, Sung J, Thierry-Mieg D, Thierry-Mieg J, Thodima V, Trygg J, Vishnuvajjala L, Wang SJ, Wu J, Wu Y, Xie Q, Yousef WA, Zhang L, Zhang X, Zhong S, Zhou Y, Zhu S, Arasappan D, Bao W, Lucas AB, Berthold F, Brennan RJ, Buness A, Catalano JG, Chang C, Chen R, Cheng Y, Cui J, Czika W, Demichelis F, Deng X, Dosymbekov D, Eils R, Feng Y, Fostel J, Fulmer-Smentek S, Fuscoe JC, Gatto L, Ge W, Goldstein DR, Guo L, Halbert DN, Han J, Harris SC, Hatzis C, Herman D, Huang J, Jensen RV, Jiang R, Johnson CD, Jurman G, Kahlert Y, Khuder SA, Kohl M, Li J, Li L, Li M, Li QZ, Li S, Li Z, Liu J, Liu Y, Liu Z, Meng L, Madera M, Martinez-Murillo F, Medina I, Meehan J, Miclaus K, Moffitt RA, Montaner D, Mukherjee P, Mulligan GJ, Neville P, Nikolskaya T, Ning B, Page GP, Parker J, Parry RM, Peng X, Peterson RL, Phan JH, Quanz B, Ren Y, Riccadonna S, Roter AH, Samuelson FW, Schumacher MM, Shambaugh JD, Shi Q, Shippy R, Si S, Smalter A, Sotiriou C, Soukup M, Staedtler F, Steiner G, Stokes TH, Sun Q, Tan PY, Tang R, Tezak Z, Thorn B, Tsyganova M, Turpaz Y, Vega SC, Visintainer R, von Frese J, Wang C, Wang E, Wang J, Wang W, Westermann F, Willey JC, Woods M, Wu S, Xiao N, Xu J, Xu L, Yang L, Zeng X, Zhang J, Zhang L, Zhang M, Zhao C, Puri RK, Scherf U, Tong W, Wolfinger RD; MAQC Consortium (2010) The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. *Nat Biotech* **28**, 827-838.

[31] Shi L, Campbell G, Jones W, Campagne F, Consortium AM (2010) The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. *Nat Biotechnol* **28**, 827-838.

[32] Corder E, Saunders A, Strittmatter W, Schmechel D, Gaskell P, Small G, Roses A, Haines J, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921-923.

[33] Westin K, Buchhave P, Nielsen H, Minthon L, Janciauskiene S, Hansson O (2012) CCL2 is associated with a faster rate of cognitive decline during early stages of Alzheimer's disease. *PLoS One* **7**, e30525.

[34] Chiasserini D, Parnetti L, Andreasson U, Zetterberg H, Giannandrea D, Calabresi P, Blennow K (2010) CSF levels of heart fatty acid binding protein are altered during early phases of Alzheimer's disease. *J Alzheimers Dis* **22**, 1281-1288.

[35] Olsson B, Hertze J, Ohlsson M, Nägga K, Höglund K, Basun H, Annas P, Lannfelt L, Andreasen N, Minthon L (2013) Cerebrospinal fluid levels of heart fatty acid binding protein are elevated prodromally in Alzheimer's disease and vascular dementia. *J Alzheimers Dis* **34**, 673-679.

[36] Kester MI, Teunissen CE, Crimmins DL, Herries EM, Ladenson JH, Scheltens P, van der Flier WM, Morris JC, Holtzman DM, Fagan AM (2015) Neurogranin as a cerebrospinal fluid biomarker for synaptic loss in symptomatic Alzheimer disease. *JAMA Neurol* **72**, 1275-1280.

[37] Craig-Schapiro R, Kuhn M, Xiong C, Pickering EH, Liu J, Misko TP, Perrin RJ, Bales KR, Soares H, Fagan AM (2011) Multiplexed immunoassay panel identifies novel CSF biomarkers for Alzheimer's disease diagnosis and prognosis. *PLoS One* **6**, e18850.

[38] Cai WW, Wang L, Chen Y (2010) Aspartyl aminopeptidase, encoded by an evolutionarily conserved syntenic gene, is colocalized with its cluster in secretory granules of pancreatic islet cells. *Biosci Biotechnol Biochem* **74**, 2050-2055.

[39] Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A (2015) Tissue-based map of the human proteome. *Science* **347**, 1260419.

[40] Xie J, Zhang B, Lan MS, Notkins AL (1998) Genomic structure and promoter sequence of the insulin-dependent diabetes mellitus autoantigen, IA-2 (PTPRN). *Genomics* **54**, 338-343.

[41] Saeki K, Zhu M, Kubosaki A, Xie J, Lan MS, Notkins AL (2002) Targeted disruption of the protein tyrosine phosphatase-like molecule IA-2 results in alterations in glucose tolerance tests and insulin secretion. *Diabetes* **51**, 1842-1850.

[42] Nishimura T, Kubosaki A, Ito Y, Notkins AL (2009) Disturbances in the secretion of neurotransmitters in IA-2/IA-2β null mice: Changes in behavior, learning and lifespan. *Neuroscience* **159**, 427-437.

[43] Kuusisto J, Koivisto K, Mykkänen L, Helkala E-L, Vanhanen M, Hänninen T, Kervinen K, Kesäniemi YA, Riekkinen PJ, Laakso M (1997) Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: Cross sectional population based study. *BMJ* **315**, 1045-1049.

[44] Matsuzaki T, Sasaki K, Tanizaki Y, Hata J, Fujimi K, Matsui Y, Sekita A, Suzuki S, Kanba S, Kiyohara Y (2010) Insulin resistance is associated with the pathology of Alzheimer disease The Hisayama Study. *Neurology* **75**, 764-770.

[45] Schrijvers EM, Witteman J, Sijbrands E, Hofman A, Koudstaal PJ, Breteler M (2010) Insulin metabolism and the risk

of Alzheimer disease: The Rotterdam Study. *Neurology* **75**, 1982-1987.

[46] Steen E, Terry BM, J Rivera E, Cannon JL, Neely TR, Tavares R, Xu XJ, Wands JR, de la Monte SM (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *J Alzheimers Dis* **7**, 63-80.

[47] Kandimalla R, Thirumala V, Reddy PH (2017) Is Alzheimer's disease a type 3 diabetes? A critical appraisal. *Biochim Biophys Acta* **1863**, 1078-1089.

[48] Hokama M, Oka S, Leon J, Ninomiya T, Honda H, Sasaki K, Iwaki T, Ohara T, Sasaki T, LaFerla FM (2013) Altered expression of diabetes-related genes in Alzheimer's disease brains: The Hisayama study. *Cereb Cortex* **24**, 2476-2488.

[49] Antonell A, Lladó A, Altirriba J, Botta-Orfila T, Balasa M, Fernández M, Ferrer I, Sánchez-Valle R, Molinuevo JL (2013) A preliminary study of the whole-genome expression profile of sporadic and monogenic early-onset Alzheimer's disease. *Neurobiol Aging* **34**, 1772-1778.

[50] Silva AR, Grinberg LT, Farfel JM, Diniz BS, Lima LA, Silva PJ, Ferretti RE, Rocha RM, Jacob Filho W, Carraro DM (2012) Transcriptional alterations related to neuropathology and clinical manifestation of Alzheimer's disease. *PLoS One* **7**, e48751.

[51] Sun Y, Nilsson M, Salter H (2013) Genetic interaction analysis of Alzheimer's disease progression using phospho-tau as a covariate. *Alzheimers Dement* **9**, P555-P556.

[52] Hu WT, Chen-Plotkin A, Arnold SE, Grossman M, Clark CM, Shaw LM, Pickering E, Kuhn M, Chen Y, McCluskey L (2010) Novel CSF biomarkers for Alzheimer's disease and mild cognitive impairment. *Acta Neuropathol* **119**, 669-678.

[53] Gonzalez H, Ottervald J, Nilsson KC, Sjögren N, Miliotis T, Von Bahr H, Khademi M, Eriksson B, Kjellström S, Vegvari A (2009) Identification of novel candidate protein biomarkers for the post-polio syndrome—implications for diagnosis, neurodegeneration and neuroinflammation. *J Proteomics* **71**, 670-681.

[54] Yin GN, Lee HW, Cho J-Y, Suk K (2009) Neuronal pentraxin receptor in cerebrospinal fluid as a potential biomarker for neurodegenerative diseases. *Brain Res* **1265**, 158-170.

[55] Abdi F, Quinn JF, Jankovic J, McIntosh M, Leverenz JB, Peskind E, Nixon R, Nutt J, Chung K, Zabetian C (2006) Detection of biomarkers with a multiplex quantitative proteomic platform in cerebrospinal fluid of patients with neurodegenerative disorders. *J Alzheimers Dis* **9**, 293-348.

[56] Ringman JM, Schulman H, Becker C, Jones T, Bai Y, Immermann F, Cole G, Sokolow S, Gylys K, Geschwind DH (2012) Proteomic changes in cerebrospinal fluid of presymptomatic and affected persons carrying familial Alzheimer disease mutations. *Arch Neurol* **69**, 96-104.

[57] Wildsmith KR, Schauer SP, Smith AM, Arnott D, Zhu Y, Haznedar J, Kaur S, Mathews WR, Honigberg LA (2014) Identification of longitudinally dynamic biomarkers in Alzheimer's disease cerebrospinal fluid by targeted proteomics. *Mol Neurodegener* **9**, 22.

[58] Khan W, Aguilar C, Kiddle SJ, Doyle O, Thambisetty M, Muehlboeck S, Sattlecker M, Newhouse S, Lovestone S, Dobson R (2015) A subset of cerebrospinal fluid proteins from a multi-analyte panel associated with brain atrophy, disease classification and prediction in Alzheimer's disease. *PLoS One* **10**, e0134368.

[59] Lehallier B, Essioux L, Gayan J, Alexandridis R, Nikolcheva T, Wyss-Coray T, Britschgi M (2016) Combined plasma and cerebrospinal fluid signature for the prediction of midterm progression from mild cognitive impairment to Alzheimer disease. *JAMA Neurol* **73**, 203-212.

[60] Guo L-H, Alexopoulos P, Perneczky R (2013) Heart-type fatty acid binding protein and vascular endothelial growth factor: Cerebrospinal fluid biomarker candidates for Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci* **263**, 553-560.

[61] Jack Jr. CR, Wiste HJ, Vemuri P, Weigand SD, Senjem ML, Zeng G, Bernstein MA, Gunter JL, Pankratz VS, Aisen PS (2010) Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. *Brain* **133**, 3336-3348.

[62] Bouwman F, Schoonenboom S, van Der Flier W, Van Elk E, Kok A, Barkhof F, Blankenstein M, Scheltens P (2007) CSF biomarkers and medial temporal lobe atrophy predict dementia in mild cognitive impairment. *Neurobiol Aging* **28**, 1070-1074.

[63] Davatzikos C, Bhatt P, Shaw LM, Batmanghelich KN, Trojanowski JQ (2011) Prediction of MCI to AD conversion, via MRI, CSF biomarkers, and pattern classification. *Neurobiol Aging* **32**, 2322. e2319-2322. e2327.

[64] Vemuri P, Wiste H, Weigand S, Shaw L, Trojanowski J, Weiner M, Knopman DS, Petersen RC, Jack C (2009) MRI and CSF biomarkers in normal, MCI, and AD subjects: Predicting future clinical change. *Neurology* **73**, 294-301.

[65] Nesteruk M, Nesteruk T, Styczyńska M, Mandecka M, Barczak A, Barcikowska M (2016) Combined use of biochemical and volumetric biomarkers to assess the risk of conversion of mild cognitive impairment to Alzheimer's disease. *Folia Neuropathol* **54**, 369-374.

[66] Frölich L, Peters O, Lewczuk P, Gruber O, Teipel SJ, Gertz HJ, Jahn H, Jessen F, Kurz A, Luckhaus C (2017) Incremental value of biomarker combinations to predict progression of mild cognitive impairment to Alzheimer's dementia. *Alzheimers Res Ther* **9**, 84.