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Imaging Proteomics for Diagnosis, Monitoring and Prediction of Alzheimer's Disease

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Abstract

Proteomic and imaging markers have been widely studied as potential biomarkers for diagnosis, monitoring and prognosis of Alzheimer's disease. In this study, we used Alzheimer Disease Neuroimaging Initiative dataset and performed parallel independent component analysis on cross sectional and longitudinal proteomic and imaging data in order to identify the best proteomic model for diagnosis, monitoring and prediction of Alzheimer disease (AD).

We used plasma proteins measurement and imaging data from AD and healthy controls (HC) at the baseline and 1 year follow-up. Group comparisons at baseline and changes over 1 year were calculated for proteomic and imaging data. The results were fed into parallel independent component analysis in order to identify proteins that were associated with structural brain changes cross sectionally and longitudinally. Regression model was used to find the best model that can discriminate AD from HC, monitor AD and to predict MCI converters from non-converters.

We showed that five proteins are associated with structural brain changes in the brain. These proteins could discriminate AD from HC with 57% specificity and 89% sensitivity. Four proteins whose change over 1 year were associated with brain structural changes could discriminate AD from HC with sensitivity of 93%, and specificity of 92%. This model predicted MCI conversion to AD in 2 years with 94% accuracy. This model has the highest accuracy in prediction of MCI conversion to AD within the ADNI-1 dataset. This study shows that combination of selected plasma protein levels and MR imaging is a useful method in identifying potential biomarker.

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Keywords

Alzheimer’s Disease; Tensor Based Morphometry; Proteomics; Magnetic Resonance Imaging; Biomarkers; Parallel ICA

Introduction

Alzheimer’s disease (AD) is the most common neurodegenerative disorder. Emerging diagnostic techniques using multiple imaging modalities have contributed tremendously to our knowledge of the disease in recent years (Perrin et al., 2009), but AD’s pathophysiology still remains largely elusive. Emerging disease-modifying strategies for AD necessitate accurate biomarkers for early diagnosis, monitoring and prognosis more than ever before. Magnetic resonance imaging (MRI) measures have been shown to predict conversion of mild cognitive impairment (MCI) to AD (deToledo-Morrell et al., 2004; Jack et al., 1999; Risacher et al., 2010; Whitwell et al., 2008) and cognitive decline in elderly (Mungas et al., 2002; Rusinek et al., 2003). Similarly proteomic indices have been shown to have diagnostic value in AD (Britschgi and Wyss-Coray, 2009; Hye et al., 2006; Ray et al., 2007) and predict MCI conversion to AD (Ray et al., 2007). There are potential advantages of proteomics over imaging as biomarkers in AD. For example, a recent study on familial AD estimated that changes in Aβ amyloid and tau protein levels may occur 5 years before brain atrophy is detectable in MRI (Bateman, 2012). A group of investigators recently used multivariate approach for analysing proteomic profile in Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset and found that apoE, B-type natriuretic peptide, C-reactive protein, and pancreatic polypeptide are significantly different between MCI and AD group (Hu et al., 2012). They also showed that CSF Aβ42 levels and t-tau/Aβ42 ratios correlated with the number of apoE4 alleles, plasma levels of B-type natriuretic peptide and pancreatic polypeptide (Hu et al., 2012). However it is not clear if any of these proteins are associated with changes in the brain, as they did not use any imaging data in their analysis.

Combining imaging markers with proteomics (i.e. Imaging Proteomics) provides an opportunity to identify proteins that are specifically associated with changes in the brain (Mattay et al., 2008; Meyer-Lindenberg and Weinberger, 2006). In addition, this approach may provide further mechanistic insights into pathophysiological mechanisms of AD, which in turn could serve as new targets for therapeutic strategies (Britschgi and Wyss-Coray, 2009).

Data mining in a large dataset with many different variables is prone to the problem of over fitting, and is all the more challenging due to the within-modality correlations with mass univariate techniques (Pearlson, 2009). Simply reducing the number of features considered does not necessarily address the problem, as the relationship between variables may also provide crucial information; for example, the discovery of Metabolic Syndrome X (Eckel et al., 2005) was based on a set of closely correlated physiological variables.

Parallel independent component analysis (PICA) greatly diminishes this methodological problem (Liu et al., 2008). PICA is an unsupervised multivariate algorithm to extract independent within-modality patterns with strongest between-modality connections when

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more than one modality is available. The intermodal associations between resulting components are then further identified and quantified. These components can be later compared on a component-wise basis between experimental groups. PICA has been applied widely in neurosciences research such as assessing relationships between electroencephalography (EEG), structural and functional MRI (fMRI), with single-nucleotide polymorphism (SNP) array (Jagannathan et al., 2010; Liu et al., 2009a; Liu et al., 2009b), EEG with fMRI (Wu et al., 2010) and positron emission tomography (PET) with structural MRI (Tosun et al., 2011). Here, we used PICA to explore the relationship between structural MRI data and proteomics in ADNI dataset. The goal was to determine proteins that were associated with brain structural changes and therefore could have potential value as biomarkers for diagnosis and monitoring AD as well as predicting MCI conversion to AD.

Methods

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date these three protocols have recruited over 1500 adults, ages 55 to 90, to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2 and ADNI-GO. Subjects originally recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see www.adni-info.org. Here, we used ADNI-1 and ADNI Plasma QC Multiplex Data.

Subjects

The eligibility criteria for the inclusion of participants are described at: http://adni.loni.ucla.edu/wp-content/uploads/2010/09/ADNI_GeneralProceduresManual.pdf. Subjects are divided into the following groups:

Healthy Controls (HC): Mini-Mental State Examination (MMSE) scores between 24-30, Clinical Dementia Rating (CDR) of 0, non-depressed and non-MCI.

MCI subjects: MMSE scores between 24-30, with memory complaint, objective memory loss measured by education adjusted scores on Wechsler Memory Scale Logical Memory II, a CDR of 0.5, no significant levels of impairment in other cognitive domains, and preserved activities of daily living.

Mild AD subjects: MMSE scores between 20-26, CDR of 0.5-1.0, meeting NINCDS/ADRDA criteria for probable AD (McKhann et al., 1984).

In this study, we chose AD and HC subjects from ADNI database who had baseline and 12-month-follow-up plasma proteins measurement and had pre-processed quality checked structural MRI data. For the MCI patients, only those who had baseline plasma proteins levels and had been followed up for at least two years were selected (n=300), of whom 110 subjects converted to AD during the follow up period.

Targeted Multiplex Proteomics

Procedure of plasma protein data collection and measurement is explained in detail elsewhere (http://adni.loni.ucla.edu/wp-content/uploads/2010/11/BC_Plasma_Proteomics_Data_Primer.pdf). In brief, plasma proteins were measured in a subset of EDTA plasma samples (obtained in the morning following an overnight fast) at baseline and 1 year follow up, using a 190 analyte multiplex immunoassay panel. The panel, referred to as the human discovery map, was developed on the Luminex xMAP platform by Rules-Based Medicine (RBM) to contain proteins previously reported in the literature to be altered as a result of cancer, cardiovascular disease, metabolic disorders, and inflammation. In addition, RBM partnered with Satoris (Inc., California, USA) to include plasma proteins previously reported to change in patients with Alzheimer's disease (Ray et al., 2007). Assays have been qualified based on the least detectable dose, precision, cross-reactivity, dilutional linearity, and spike recovery. Results of analyses on 148 analytes, which passed quality control, were used in this study.

MRI Acquisition and Preprocessing

High-resolution T1-weighted MRI scans were acquired on 1.5 Tesla MRI scanners from Siemens, General Electric Healthcare, and Philips Medical Systems with the standard ADNI MRI protocol (Jack et al., 2008). Each subject was scanned with a sagittal 3D MP-RAGE sequence, with acquisition parameters: inversion time (TI)/repetition time (TR): 1000/2400 ms; flip angle: 8°; 24 cm field of view; 192×192×166 acquisition matrix, and a voxel size of 1.25× 1.25×1.2 mm^3. In plane, zero-filled reconstruction yielded a 256×256 matrix for a reconstructed voxel size of 0.94×0.94×1.20 mm^3, later reconstructed to 1 mm isotropic voxels. The scan quality was evaluated by the ADNI MRI quality control centre at the Mayo Clinic following standardized criteria. Images were calibrated with phantom-based geometric corrections to ensure consistency among scans acquired at different sites. Image corrections were applied using a processing pipeline at the Mayo Clinic, consisting of: (1) correction of geometric distortion due to gradient non-linearity (Jovicich et al., 2006), i.e., "gradwarp", (2) "B1 correction" for adjustment of image intensity inhomogeneity due to B1 non-uniformity (Jack et al., 2008), (3) "N3" bias field correction for reducing residual intensity inhomogeneity (Sled et al., 1998), and (4) geometrical scaling for removing

scanner and potential session specific calibration errors using a phantom scan acquired for each subject (Gunter et al., 2009). All original image files as well as images with all of these corrections are available to the general scientific community at http://www.loni.ucla.edu/ADNI/Data/.

Voxel-Based Morphometry

For the cross-sectional (baseline) study, data pre-processing and voxel-based morphometry (VBM) were performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and MATLAB v7.11 (Math-Works, Natick, MA, USA). First, tissue classification was carried out on MR images to separate gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using the standard unified segmentation model in SPM8 (Ashburner and Friston, 2005). Second, GM population templates were generated from the entire image dataset using the diffeomorphic anatomical registration using exponentiated Lie algebra (DARTEL) technique (Ashburner, 2007). Third, after an initial affine registration of the GM DARTEL templates to the tissue probability maps in Montreal Neurological Institute (MNI) space (http://www.mni.mcgill.ca/), non-linear warping of GM images was performed to the DARTEL GM template in MNI space. Fourth, images were then modulated to ensure that relative volumes of GM were preserved following the spatial normalization procedure. Finally, images were smoothed with an 8 mm full width at half maximum Gaussian kernel. To identify group differences in the modulated GM a two-sample t-test was used at each voxel. To find differences that were spatially extended, we used an inference method on the t-test images called Threshold Free Cluster Enhancement (TFCE) (Smith and Nichols, 2009). TFCE is an extension of cluster-wise inference that does not depend on a cluster-forming threshold. The risk of false positives over the brain was controlled with the family wise error rate (5% level) using a permutation method with 5000 permutations (Nichols and Holmes, 2002), (FSL version 4.1.9, http://www.fmrib.ox.ac.uk/fsl/). A binary mask was created from voxels showing significant between group differences. Pre-processed GM images and the resulting masks were used as inputs for parallel ICA.

Tensor-Based Morphometry

Tensor based morphometry (TBM) was used to assess changes over time i.e. for longitudinal analysis mainly using SPM8 software package. Pre-processing stages for TBM have been described in detail elsewhere (Kipps et al., 2005). Briefly, the follow up T1-weighted MRI image scan was co-registered with the baseline image. A high-dimensional deformation field was used to warp the corrected late image to match the baseline image for each subject (Ashburner and Friston, 2000). The amount of volume change was quantified by taking the determinant of the gradient of deformation at the single-voxel level (*Jacobian determinants*). The following formula was applied to the segmented gray matter image obtained from the first scan and the Jacobian determinant map: $(Jacobian\ value-1) \times GM$. The resulting product image represented a measure of the gray matter specific volume change between the baseline and follow up scans. The normalization parameters were estimated by matching the customized gray matter template that was created earlier with the segmented gray matter image from the first scan and were then applied to the product image (Ashburner and Friston, 1999). Normalized images were smoothed using an 8 mm isotropic Gaussian kernel. Finally, the smoothed images were multiplied by an inclusive gray matter mask. The value

of each voxel in pre-processed image shows volume loss or expansion during 1-year follow up. Statistical analysis was performed using permutation testing as described above. Binary mask was created from voxels showing significant group difference (AD<HC) in the pre-processed images ($p < 0.05$ FWE-corrected) during one year. The resulting binary mask and pre-processed images from one year longitudinal TBM were used as inputs for monitoring disease progression (see below).

Parallel Independent Component Analysis (PICA)

We used PICA to explore the relationship between plasma proteins and regional brain atrophy simultaneously. The details of PICA has been described elsewhere (Liu et al., 2009b). Briefly ICA estimates sets of variables (components) that are maximally independent; specifically, it minimizes the mutual information between pairs of components. PICA, for two modalities, simultaneously optimizes the independence of components in each modality *and* the similarity of subject weights between the modalities. Thus two sets of components are produced; one for each modality, but a subset of the components will be defined by subject weights that are similar between the two modalities (Liu et al., 2009b). The number of components in each analysis was estimated by Akaike Information Criterion and Minimum Distance Length (Calhoun et al., 2001). All of the components were normalized to Z score to remove scaling and thresholded at $z > 1.7$ for visualization purposes. The Pearson correlation coefficients were computed for every possible pair of subject weights between the two modalities, and significant relationships determined using false discovery rate ($P_{FDR} < 0.05$).

Following steps of analyses (see Figure 1) were carried out:

1- To identify proteomic makers that may have potential value as biomarkers for diagnosis of AD, we first used a two-sample t-test to find significant differences (increase or decrease) in plasma protein concentration between AD and HC groups as well as in their GM density separately. Then PICA was run on those proteins and brain regions that showed significant differences (increase or decrease) between groups (after correction for multiple comparisons, FWE for images, and FDR for proteomics. The result provides proteins that are closely associated with modulated GM at the baseline.

2- To identify proteins that may have potential value as biomarkers for monitoring AD progression, we first calculated TBM maps of AD and HC subjects over one year showing longitudinal structural brain changes. We then calculated plasma concentration difference (increase or decrease) of the proteins for each individual over one year. A two-sample t-test on the longitudinal protein concentration changes and TBM results to identify between groups difference (corrected with FWE, $p<0.05$). Finally PICA was run on the TBM and protein changes (increase or decrease) over 1 year in AD subjects only. This analysis identified proteins whose changes over one year correlated with the structural changes of the brain in AD.

3- To identify proteins that may have potential value as biomarkers to determine the prognosis of MCI, we first carried out logistic regression (using stepwise

forward Wald method) on the resulted proteomic factors of the cross-sectional analysis and corrected that for age and sex, to develop the best model for discriminating AD from HC participants. The rational for this model was that proteins that strongly correlated with brain structural changes were likely to be useful potential biomarkers for diagnosis of AD. In addition, protein changes might precede brain structural changes or/and cognitive changes. Also, we aimed to produce a totally data-driven model rather than using a priori. A similar approach was applied to the longitudinal data. We used this model to identify MCI subjects who converted to AD. Receiver operating characteristic (ROC) curves was graphed and areas under the curves (AUC) along with its 95% confidence intervals (CI) were computed. Note that MCI subjects were not used for developing the "predictive" model to obtain unbiased estimates of MCI conversion accuracy.

Results

Data from total of 49 HC, 300 MCI (of which 110 converted to AD within 2 years), and 85 AD patients were analysed. Participants' demographic and cognitive data are summarized in Table 1. Schematic presentation of steps of analysis and the results is shown in Figure 1. Plasma level of 18 proteins that showed cross sectional group difference at the baseline and 64 proteins that showed significant longitudinal difference between groups in a year are shown in Table S1, S2, S3, S4 (supplementary data).

Cross-Sectional Analysis at Baseline

Eighteen proteins exhibited significant differences ($p < 0.05$, FDR-corrected) between AD and HC subjects (Table S1). The cross-sectional VBM analysis found significantly reduced ($p<0.05$, FWE-corrected) gray matter in AD subjects relative to HC in several brain regions, including hippocampus, amygdala, thalamic nuclei, posterior cingulate-precuneus junctions, inferior, middle and superior temporal gyri, parahippocampal/fusiform cortices and right angular gyrus (Figure 2). PICA showed that 8 imaging components (components that were resulted from running ICA on imaging data) associated with 9 protein components (Table S2). Of the 8×9=72 possible intermodal pairwise correlations, 5 components had statistically significant relationship ($P_{FDR} < 0.05$) (Figure 2). Modulated GM in hippocampus, amygdala and several other temporal lobe structures along with posterior cingulate cortex and thalamus was associated with decreased interleukin-16 (IL-16), and apolipoproteinE (apoE) as well as increased thyroxin-binding globulin (TBG), and alpha-2-macroglobulin levels. Modulated GM in right inferior and middle temporal gyri was associated with increased brain natriuretic peptide (BNP), pancreatic polypeptide (PPP), and peptide YY (PYY) concentrations. Thalamic GM was significantly correlated with decreased IgM, as well as increased Eotaxin-3, and PYY levels. Among the 5 significant intermodal components, a total of 9 proteins had significant Z-scores ($P_{FDR} < 0.05$). The proteins and brain regions in these components are summarized in Table 2.

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Longitudinal Analysis

sixty-four proteins showed significant difference in their changes over 1 year in AD when compared with controls ($P_{FDR} < 0.05$) (Table S1). Similarly, caudate, putamen, amygdala, fusiform cortex, posterior cingulate, lingual, and parahippocampal gyri, and right anterior cingulate and right middle temporal gyri showed significantly ($P_{FWE} < 0.05$) more gray matter loss in AD than healthy subjects over 1 year follow up (Figure 3). PICA on AD subjects (n=85) identified 10 proteomic components associated with 12 structural imaging components (Table S1, S3 and S5). Of the 10×12=120 possible intermodal pairwise correlations, 4 components were significant ($P_{FDR} < 0.05$) (Figure 3). In AD, right fusiform cortex atrophy rate was associated with decreased bone morphogenetic protein 6 (BMP6), and fibroblast growth factor 4 (FGF4), while these factors increased in healthy controls. Right fusiform atrophy was also associated with less reduction in AD (compared to normal reduction) of neuronal cell adhesion molecule (NCAM), and matrix metalloproteinase-10 (MMP-10). In addition, increased matrix metalloproteinase-9 (MMP-9), and superoxide dismutase 1- soluble (SOD-1) levels were associated with right fusiform atrophy. Cingulate gyrus and left fusiform cortex atrophy rate were associated with more reduction (compared with normal reduction) of von willebrand factor (vWF) level. Atrophy rate of amygdala was associated with reduced E-selectin level (Table 2). Among the 4 significant intermodal components, a total of 8 proteins had significant Z-scores significant ($P_{FDR} < 0.05$).

Diagnostic and Prognostic Utility of the Potential Biomarkers

The 9 proteins, which significantly correlated with regional brain atrophy at the baseline, were entered into the stepwise logistic regression and 5 proteins were found to be capable of meaningfully classifying AD from control (Table 3). This model discriminated AD from normal subjects with specificity of 62% and sensitivity of 93% (AUC=0.917, CI=[0.86,0.96], SD=0.02, p<0.0001). Cross validation using discriminant analysis with leave one out (DALOO) showed sensitivity of 89.4%, and specificity of 57%. When this model was applied on MCI patients (n=342) at the baseline, 64.5% (40 out of 62) of converters to AD were correctly identified.

In addition, the four proteins whose changes over time correlated with structural brain changes were used as a model to discriminate AD from control. This model had 92.9% sensitivity and 69.4% specificity. Cross validation of this model using DALOO showed sensitivity of 92.9%, and specificity of 91.8%. We, then, used this model to predict MCI converters and found that the model correctly identified 93.5% (58 out of 62) of the converters. When ApoE (a well known risk factor for AD) was removed from the model, the sensitivity and specificity of the model for discriminating AD from control dropped to %85 and %50 respectively and the predictive power of MCI convertors to AD fell to 56%. Adding age and sex to the model did not affect the result significantly.

Discussion

Protein changes at baseline

There are differences in GM in AD patients relative to controls in hippocampus, amygdala, thalamic nuclei, posterior cingulate-precuneus junctions, inferior, middle and superior

temporal gyri, parahippocampal/fusiform cortices and right angular gyrus. This is consistent with previous studies (Echavarri et al., 2011; Karas et al., 2003; Shiino et al., 2006; Singh et al., 2006). We also found that plasma levels of 18 proteins were significantly changed in AD subjects when compared with control group. Among these proteins the levels of IL-16, TBG, α_2-Macroglobulin, apoE, BNP, PPP, PYY, IgM, and Eotaxin-3 were associated with regional brain atrophy at baseline. Current biological evidence supports relevance of these proteins to pathophysiology of Alzheimer disease. For example, many studies showed the role of apoE in AD particularly through its catalytic action on amyloid Aβ (Potter and Wisniewski, 2012). In our study, apoE plasma level was associated with atrophy of hippocampus, posterior cingulate, inferior and middle temporal gyri, thalamic nuclei, amygdala, temporal fusiform cortex, and parahippocampal gyri. This is consistent with previous studies showing positive correlation between apoE level and GM density (Gupta et al., 2011), and apoE level and cognitive function (Yasuno et al., 2012a). A more recent study in a non-demented elderly population showed that persons with the APOEε4 allele have a smaller hippocampal volume (den Heijer et al., 2012). Another study showed that APOEε4 carriers exhibited greater atrophy of medial temporal lobe structures (Wolk and Dickerson, 2010). A task related fMRI study on AD patients, in which subjects were asked to detect novel items from previously learned items, showed that subjects with APOEε4 demonstrated less signal change in hippocampus (Johnson et al., 2006). Plasma apoE levels were also found to correlate with Aβ burden (Gupta et al., 2011) and cognitive performance in the aging population (Yasuno et al., 2011; Yasuno et al., 2012b).

Previous studies showed α_2-Macroglobulin (A2M) increase in plasma (Hye et al., 2006) and its association with neural metabolism in hippocampus (Thambisetty et al., 2008) of patients with AD. In our study, association of A2M with atrophy of medial temporal lobe structures may be a representation of amyloid deposition and subsequent cell loss in these areas that often occur in AD. There are also independent studies showing A2M elevation in temporal cortex of AD patients (Wood et al., 1993). A2M is induced by inflammatory cytokines released by neural cells (Strauss et al., 1992) and is a marker for brain blood barrier breakdown (Cucullo et al., 2003). In addition, we found that IL-16 level was associated with atrophy of hippocampus, posterior cingulate, inferior and middle temporal gyri, and thalamus. Together these findings support an inflammatory process in early AD, which may be a useful target for future therapies. Interestingly, plasma IL-16 was reported to increase in mild and moderate AD but not in severe AD cases (Motta et al., 2007), suggesting that these proteins may only be useful markers at the early stages of the disease.

IgM, another inflammatory marker, was increase in AD group. Increased IgM in patients with AD has been previously reported (Kell et al., 1996; Soares et al., 2012) however there is no evidence to support the specificity of IgM for a pathologic process in thalamus in AD. IgM increase may be an epiphenomenon related to an inflamatory or infectious process in AD subjects (Miklossy, 2008).

We found that BNP level was associated with right inferior and middle temporal gyri atrophy. We found no evidence in the literature to support a specific link between BNP and these structures. However, a previous report showed independent association of raised N-terminal pro-hormone of BNP (NT-proBNP) level with poor cognitive performance, even in

subjects with no history of prior stroke or coronary heart disease (Daniels et al., 2011). BNP is co-secreted from strained cardiac myocytes and has been associated with congestive heart failure. A study on a cohort of dementia showed that high plasma BNP levels was associated with vascular dementia, whereas they failed to demonstrate a significant increase in the AD subjects (Kondziella et al., 2009). Using a multiplex platform, it was shown that elevated NT-proBNP in CSF could discriminate mildly demented subjects from cognitively normal controls (Craig-Schapiro et al., 2011). Our findings indicate that BNP may not be a specific marker for AD (Soares et al., 2012) but it might indicate a vascular component for AD development (Zlokovic, 2011).

Thyroxine-Binding Globulin is produced by the liver and binds thyroxine in blood. TBG level change may be associated with thyroid dysfunction that is common in the elderly. Low and high tertile of serum thyrotropin concentration has been shown to increase risk of Alzheimer disease in women (Tan et al., 2008).

Our study showed that pancreatic peptides and Eotaxin-3 significantly change in AD patients. This is consistent with previous reports (Craig-Schapiro et al., 2011; Koide et al., 1995; Soares et al., 2012; Westin et al., 2012). However, we found no evidence to support specific contribution of pancreatic peptides and Eotaxin-3 in thalamic pathology in AD (as showed in our study). Reduction of pancreatic peptides binding sites have been reported in the temporal cortex and hippocampus of AD subjects (Martel et al., 1990), though there are reports against pancreatic peptide changes in AD (Wikkelso et al., 1991). Recently, it was shown that increased CSF concentration of Eotaxin-3 was associated with conversion of MCI to AD (Westin et al., 2012). Eotaxin-3, along with other CSF biomarkers, could enhance power of tau/Aβ42 ratio to discriminate between subjects with CDR=0 and CDR>0 (Craig-Schapiro et al., 2011). Others showed correlation of CSF Aβ42 levels and t-tau/Aβ42 ratios with the number of apoE4 alleles, plasma levels of B-type natriuretic peptide and pancreatic polypeptide have bee demonstrated (Hu et al., 2012). Our study partly confirmed the result of the latter study however there are difference in the methodology. For example, we only identified proteins that are significantly associated with brain structural changes therefore reducing the chance of epiphenomena in our result.

Taking together, current available evidence is not sufficient to suggest that these peptides would be useful potential biomarkers for detection of AD.

Protein Interval Changes

We found that plasma level of 64 proteins changed within 1 year in AD more than that seen in controls. However, only changes of eight proteins were associated with regional brain atrophy including the right and left fusiform cortices, cingulate gyrus and amygdala. These proteins were BMP6, MMP-9, NCAM, FGF4, MMP-10, SOD-1, vWF, and E-selectin. Previous reports showed that BMP6, MMP-9, FGF4, NCAM and SOD-1 were linked to regulation of adult neurogenesis (Boutin et al., 2009; Crews et al., 2010; Faiz et al., 2006; Ingraham et al., 2011; Kosaka et al., 2006), a neural replenishment mechanism that is believed to be impaired in AD (Lazarov et al., 2010). Moreover, it has been shown that MMP-9 and SOD-1 have anti-Aβ amyloid properties (Murakami et al., 2011; Yan et al., 2006). Several previous studies found no significant difference in plasma MMP-9 level

between healthy controls and AD patients at baseline (Lim et al., 2011; Lorenzl et al., 2003; Martin-Aragon et al., 2009), whereas one study reported significantly decreased MMP-9 in AD (Horstmann et al., 2010) and another showed that MMP-9/biliverdin reductase ratio could predict MCI to AD progression (Mueller et al., 2010). It has been demonstrated that CSF concentrations of MMP-10 in association with that of Eotaxin-3, could enhance differentiation of demented patients from non-demented healthy controls (Craig-Schapiro et al., 2011).

We showed that Von Willebrand factor (vWF) changes over time showed negative correlation with left fusiform atrophy and positive correlation with cingulate gyri atrophy. vWF is a marker of endothelial dysfunction (Vischer, 2006) which, in a recent meta-analysis, showed to be associated with all types of dementias particularly of vascular type (Quinn et al., 2011) supporting the notion implicating disturbance of endothelial activation in AD (Grammas, 2011; Zlokovic, 2011).

Potential proteomic biomarkers for diagnosis of AD

We showed that combination of plasma proteins levels of IL-16, TBG, BNP, PYY, and ApoE can discriminate AD patients from healthy individuals with sensitivity of 89% and specificity of 57%. However, changes of BMP6, Eselectin, MMP10 and NrCAM over a year can provide a more accurate model for AD diagnosis with 93% sensitivity and 92% specificity. This is superior to any other diagnostic test thus far, for example, when compared with CSF Aβ42 (Sensitivity 80%, specificity 82%), CSF Tau protein (Sensitivity 80%, specificity 83%), MRI (Sensitivity 83%, specificity 89%), FDG-PET (Sensitivity 90%, specificity 89%), and SPECT (Sensitivity 80%, specificity 85%).

Potential Proteomic biomarkers for predicting MCI conversion to AD

We found that combination of plasma proteins levels of IL-16, TBG, BNP, PYY, and ApoE can predict MCI converters with 62.5% accuracy. This is not better than currently available measures such as brain MRI, which has 73% sensitivity and 81% specificity. However changes in BMP6, Eselectin, MMP10 and NrCAM can predict 93.5% of MCI converters, which is superior to any marker currently available for this purpose.

These findings show a real potential for use of these proteins for early diagnosis and monitoring AD as well as predict MCI conversion to AD. Clearly blood tests are more convenient and probably cheaper than imaging for diagnosis and disease monitoring.

Conclusion

This report is the most comprehensive linkage study between proteomics and structural brain atrophy in Alzheimer’s disease. We showed that combination of selected plasma protein levels and MRI may be a useful method in identifying potential biomarker for diagnosis and monitoring of AD, as well as for predicting MCI conversion to AD. Further study is required to evaluate suitability of these proteins as biomarkers in differentiating AD from other types of dementia.

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Supplementary Material

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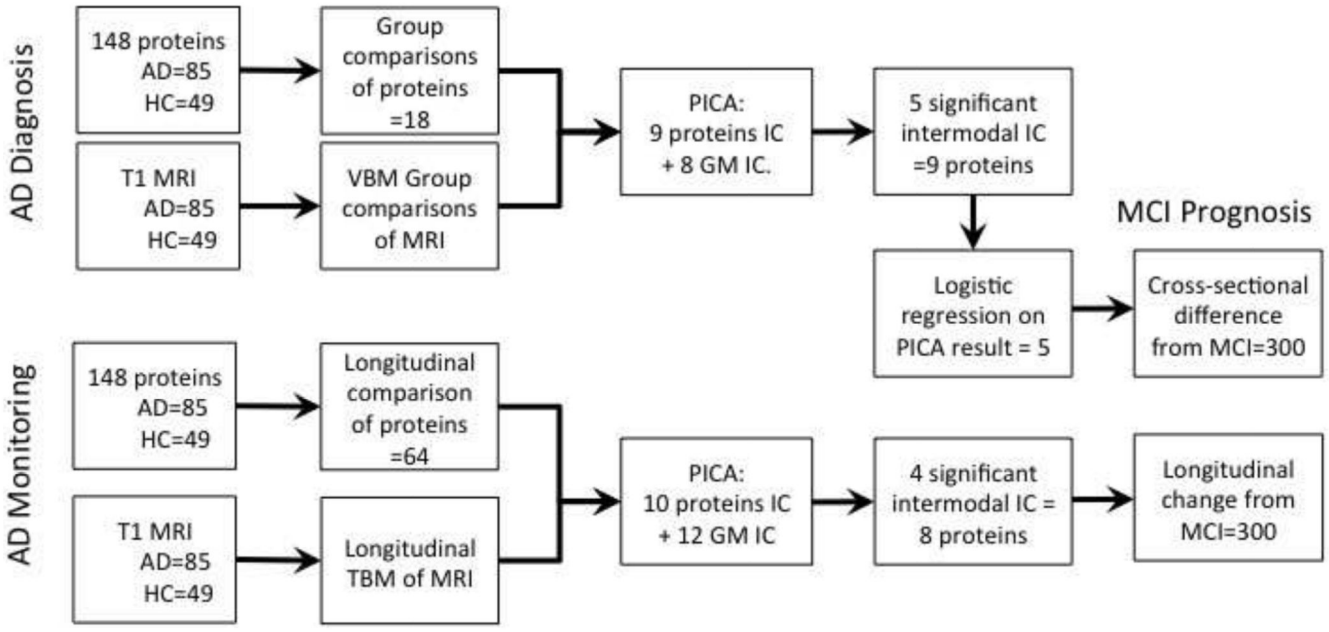

Fig.1.
Schematic representation of steps of data analysis.

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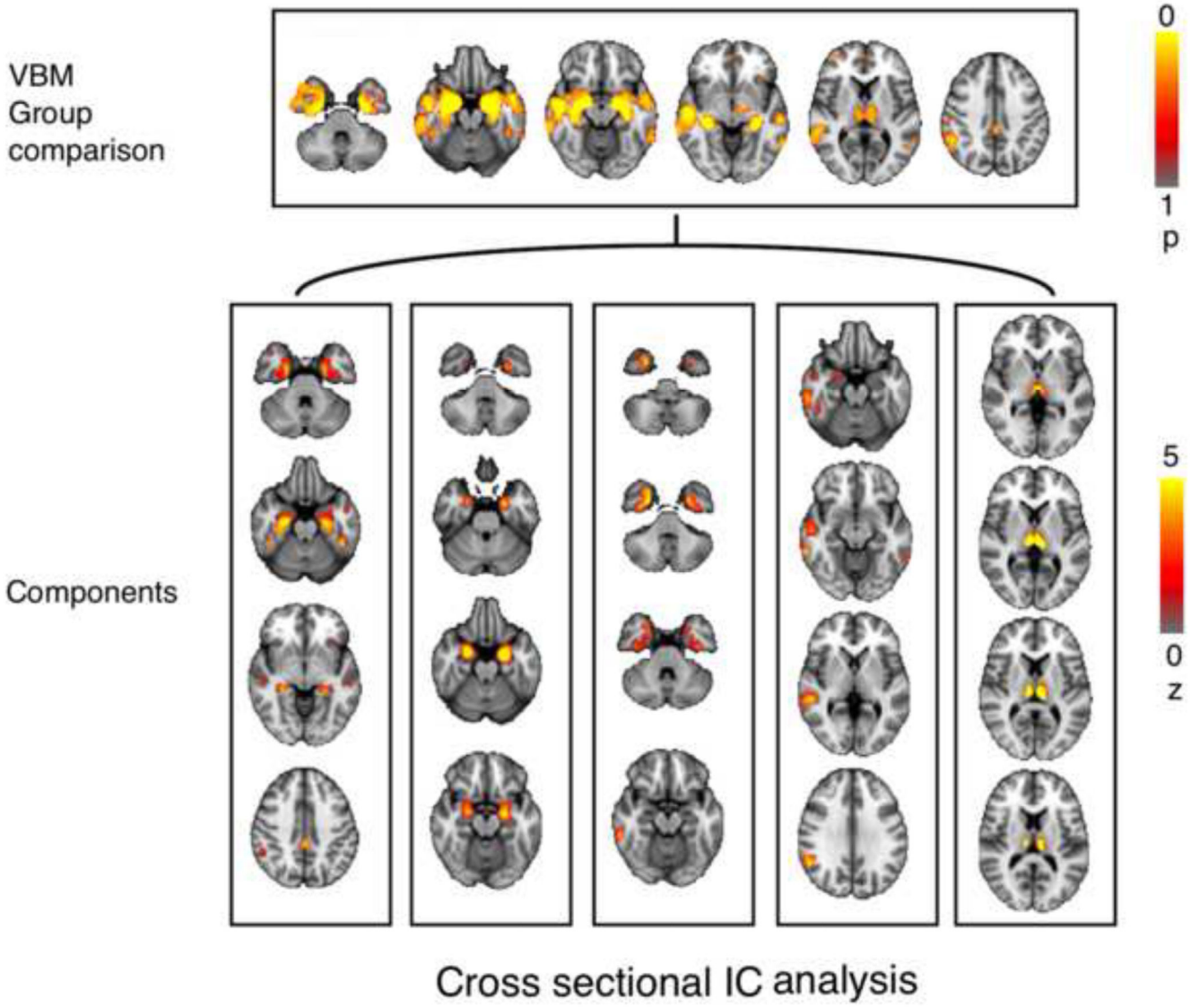

Fig 2.

Results of VBM group comparison (top) using t-test corrected for multiple comparison using TFC thresholded at p>0.05. Bottom shows the result of parallel independent component analysis at the baseline thresholded at z>1.7.

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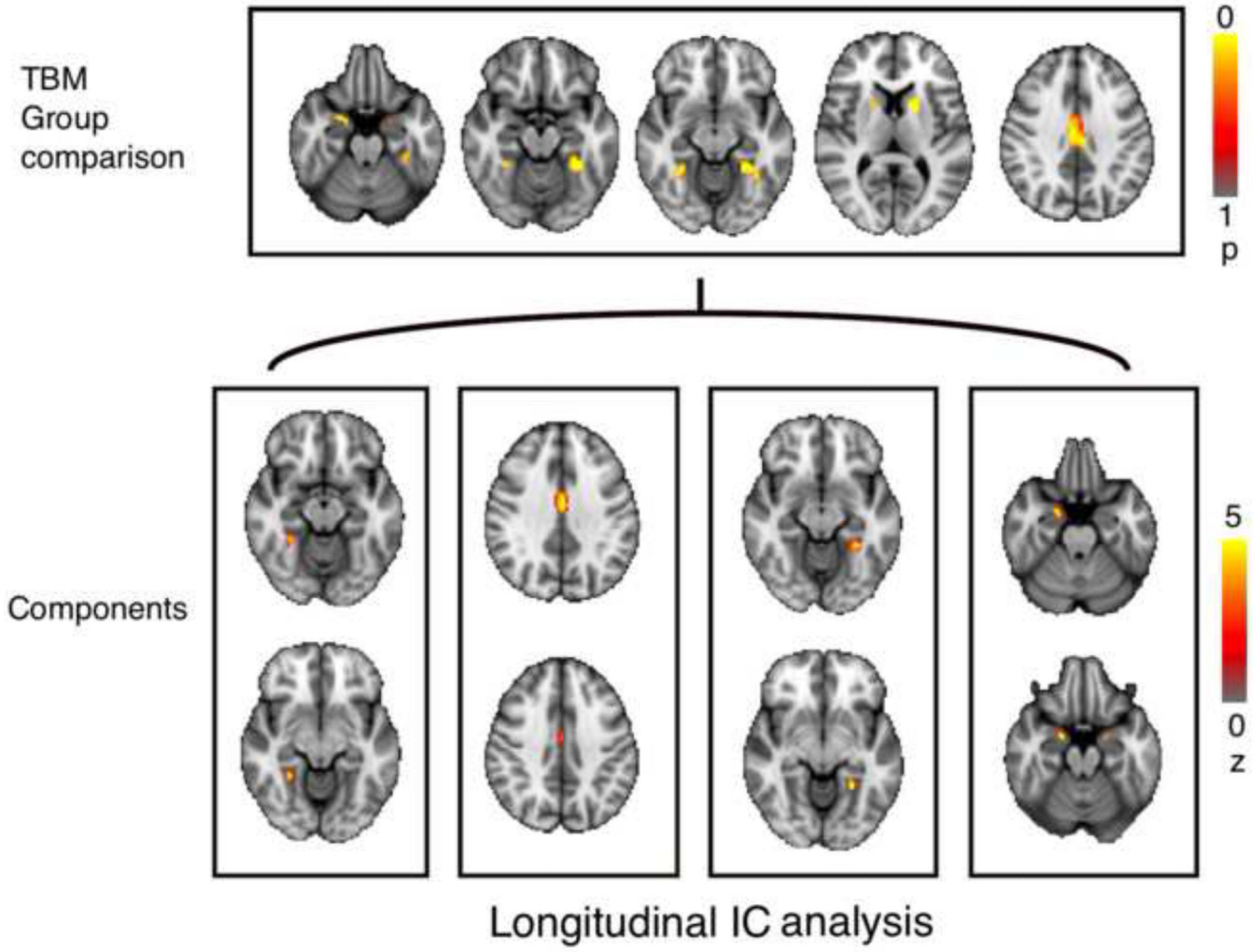

Fig 3.

Results of TBM group comparison (top) using using t-test corrected for multiple comparison using TFC thresholded at p>0.05. Bottom shows the result of parallel independent component analysis over 1 year thresholded at z>1.7.

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Table 1
Demographic and cognitive characterestics of subjects

Groups	HC	MCI	AD	F/χ^2 (p-values)
Number	49	300	85	
Age	75.1 (5.7)	74.7 (7.2)	75.2 (7.8)	0.171(0.84)
Percent Female	44.9%	35.3%	44.7%	3.73(0.15)
Education (yrs)	15.5 (2.9)	15.8 (2.9)	15.1 (3.1)	1.41 (0.24)
MMSE	29.0 (1.2)	27.1 (1.7)	23.5 (1.9)	196 (<0.001)
ADAS-Cog	9.7 (4.3)	18.2 (6.3)	28.7(7.6)	151(<0.001)

HC=Healthy Control; MCI=Mild Cognitive Impairment; AD=Alzheimer’s disease; MMSE=Mini–mental state examination; ADAS-Cog=Alzheimer's Disease Assessment Scale-cognitive subscale.

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Table 2

Plasma proteins and structural MRI components.

Study	Plasma Proteins	Z	Prot. IC	sMRI IC	Brain Regions (|z|>2.3)	r (p)
Cross-sectional	Interleukin-16	2.8	A	A	Hippocampus, Posterior Cingulate, Inferior and Middle Temporal Gyrus, Thalamic nuclei	0.41 (<0.0001)
Thyroxine-Binding Globulin	1.7	B		Amygdala, Anterior Hippocampus, Temporal Fusiform Cortex	0.30 (0.002)	
Alpha-2-Macroglobulin	1.7					
Apolipoprotein E	1.7	C		Fusiform Cortices, parahippocampal gyri, Right Inferior Temporal gyrus	0.22 (0.02)	
Brain Natriuretic Peptide	2.6	B	D	Right Inferior and middle Temporal Gyrus	-0.22 (0.02)	
Pancreatic Polypeptide	2.1					
Peptide YY	-2.0					
IgM	2.0	C	E	Thalamic Nuclei	-0.22 (0.02)	
Eotaxin-3	1.7					
Peptide YY	-1.6					
Longitudinal	Bone Morphogenetic Protein 6	4.0	A	A	Right fusiform cortex	-0.30 (0.04)
Matrix Metalloproteinase-9	3.1					
Neuronal Cell Adhesion Molecule	3.1					
Fibroblast Growth Factor 4	2.5					
Matrix Metalloproteinase-10	-2.4					
Superoxide Dismutase 1-Soluble	1.7					
von Willebrand Factor	6.9	B	B	Cingulate Gyrus	0.30 (0.04)	
			C	Left fusiform cortex	-0.27 (0.04)	
E-selectin	7.8	C	D	Amygdala	0.26 (0.04)	

Table 3
Comparing the results of different logistic regressions

	1st Logistic regression	*2nd Logistic regression*	*3rd Logistic regression*
Diagnostic	A2Macro	BNP	BNP
	Angiotensinogen	IL16	IL16
	Apo AII	PYY	PYY
	Apo B	TBG	TBG
	Apo E	APO E	APO E
	Calcitonin	PLGF	
	CK-MB	SGOT	
	Factor VII		
	FASLG Receptor		
	FSH		
	HGF		
	IL16		
	Interferon gamma		
	Induced Protein 10		
	MCP2		
	MMP9		
	PLGF		
	PYY		
	Resistin		
	SGOT		
	Vitronectin		
Sens., Spec. (DALOO)	100%, 100% (89.2%, 79%)	94.1%, 81.6% (91.8%, 81.6%)	92.9, 62.4% (89.4%, 57%)
Monitoring	BMP6	BMP6	BMP6
	B2M	Eselectin	Eselectin
	CLU	MMP10	MMP10
	MMP10	NrCAM	NrCAM
	NrCAM	Leptin	
	PLGF	PLGF	
	TFF3	TFF3	
	Thrombospondin1	VCAM	
Sens., Spec. (DALOO)	100%, 100% (94.1%, 75.5%)	100%, 100% (91.8%, 93.9%)	92.9%, 69.4% (92.9%, 91.8%)

1st Logistic regression on 148 proteins.

2nd Logistic regression on proteins that showed significant difference between groups (18 in cross sectional and 64 in longitudinal analysis). (no MRI data used)

3rd Logistic regression on proteins identified from running PICA on the result of 2nd logistic regression and between group comparisons of VBM (9 in cross sectional and 8 in longitudinal).