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Local and Distributed PiB Accumulation Associated with Development of Preclinical Alzheimer's Disease

Running head: PiB accumulation in preclinical AD

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Abstract

Amyloid-beta (Aβ) plaques are a hallmark of Alzheimer's disease (AD) that can be assessed by amyloid imaging (e.g. Pittsburgh B compound [PiB]) and summarized as a scalar value. Summary values may have clinical utility but are an average over many regions of interest, potentially obscuring important topography. This study investigates the longitudinal evolution of amyloid topographies in cognitively normal older adults who had normal (N = 131) or abnormal (N= 26) PiB scans at baseline. At 3 year follow-up, 16 participants with a previously normal PiB scan had conversion to PiB scans consistent with preclinical AD. We investigated the multivariate relationship (canonical correlation) between baseline and follow-up PiB topographies. Further, we used penalized regression to investigate the added information derived from PiB topography compared to summary measures. PiB accumulation can be local, i.e., a topography predicting the same topography in the future, and/or distributed, i.e., one topography predicting another. Both local and distributed PiB accumulation was associated with conversion of PiB status. Additionally, elements of the multivariate topography, and not the commonly used summary scalar, correlated with future PiB changes. Consideration of the entire multivariate PiB topography provides additional information regarding the development of Aβ pathology in very early preclinical AD.

Introduction

Alzheimer's disease (AD) is characterized by a long preclinical period wherein pathology accumulates in the absence of overt symptoms (Price et al., 2009). The deposition of amyloid-beta (Aβ), measured via positron emission tomography (PET) using Pittsburgh Compound B (PiB) (Klunk et al., 2004), is one of the earliest measurable pathological changes in AD (Braak and Del Tredici, 2012, Jack et al., 2013) and can be monitored longitudinally (Sojkova et al., 2011, Villemagne et al., 2011, Vlassenko et al., 2011). The results of PiB scans are often summarized into a single scalar metric defined as an average over a group of regions known to accumulate the most of Aβ plaques in symptomatic AD. PiB scans assessed in this way are dichotomized as either PiB negative (PiB–) or PiB positive (PiB+) (Mintun et al., 2006). A PiB+ scan in a cognitively normal individual is interpreted as presumptive evidence for preclinical AD (Sperling et al., 2011, Jack et al., 2012, Morris et al., 2014) and predicts clinical progression (Morris et al., 2009). Dichotomizing PiB status is potentially clinically useful but reduces complex and potentially informative topographies to a single scalar metric.

The earliest detectably abnormal amyloid topography is relatively focal but becomes more expansive as the disease progresses (Thal et al., 2002, Braak et al., 2011). Understanding of this early topography, how it differs from the topography of the highest PiB retention at the more advanced stages of AD, and how it progresses in the early stage of the disease remains unclear. Multivariate statistical techniques, well suited for examining local and distributed phenomena, may help to characterize better the relationships in longitudinal amyloid topographies as assessed by PiB.

In this study we followed 157 cognitively normal participants who were PiB– (N = 131) or PiB+ (N = 26) at baseline as assessed by a summary scalar value of mean cortical

standardized uptake value ratio (SUVR). These participants were followed longitudinally (mean follow up: ~3 years). In participants who developed significant PiB accumulation (i.e., became PiB+), we investigated the topographic progression of PiB accumulation using canonical correlation. Canonical correlation identifies pairs of highly correlated topographies. We then compared the ability of the single scalar value and topographic measures to capture the underlying PiB accumulation associated with the development of preclinical AD.

Methods

Subjects: Participants were community dwelling volunteers (age range 45-85 years) enrolled in the Adult Children Study project at the Washington University in St Louis Knight Alzheimer Disease Research Center (ADRC). All participants were cognitively normal, both at baseline and at follow-up PiB scans, as assessed by a Clinical Dementia Rating (CDR) score of 0 (Morris, 1993) and the Mini-mental status exam (MMSE). Participants were in good general health with no neurological, psychiatric, or systemic medical illness that could disrupt longitudinal participation. Each participant underwent MRI and PiB PET (described below). Baseline scans were dichotomized as either PiB– or PiB+ (criteria described below) and a second scan was performed on average 3.29 years later (standard deviation= 1.22 years; min = 0.96 years; max = 6.47 years). Participants were divided into three groups based on their PiB status at baseline and follow up: CNnn (cognitively normal, PiB– at both scans, N=115), CNnp (cognitively normal, PiB– at first scan but PiB+ at second scan, N=16), or CNpp (cognitively normal, PiB+ at both scans, N=26). Complete demographic information is shown in Table 1.

MRI Assessment: MRI consisted of an MPRAGE T1-weighted image collected on a Siemens (Erlangen, Germany) MR scanner. Images are processed using FreeSurfer software version 5.1 (Martinos Center for Biomedical Imaging, Charlestown, MA) (Fischl et al., 2002). All FreeSurfer parcelations were assessed for accuracy by a skilled investigator (KAF). Only grey matter regions were included in this analysis.

PET Assessment: The PiB PET assessment has been previously described in detail (Su et al., 2013, Su et al., 2014). Imaging was conducted on a Siemens 962 HR+ ECAT PET scanner or a Siemens Biograph 40 scanner. PET data were analyzed using previously developed methods (Su et al., 2013, Su et al., 2015). FreeSurfer segmentation (Fischl et al., 2002)

(http://freesurfer.net/) was used as the basis for quantitative analysis to obtain regional standardized uptake value ratio (SUVR) with cerebellar gray matter serving as the reference region. Partial volume correction was also performed using a regional spread function (RSF) technique (Rousset et al., 1998, Su et al., 2015).

PiB positivity was defined using the mean cortical (MC) SUVR across the precuneus, prefrontal, gyrus rectus and temporal Freesurfer ROIs (Morris et al., 2010). A cut-off value of 1.42 was used which is comparable to a mean cortical binding potential of 0.18 that was previously defined (Mintun et al., 2006, Su et al., 2013). Characteristic SUVR images are presented in Supplemental Figure 1. The equivalence for a MC-SUVR of 1.42 and MC-BP of 0.18 was previously determined using regression in an independent sample (unpublished data). Due to the criticality of this cut-off for subsequent analyses, primary results are also replicated using an alternative cut-off determined from this sample (Supplemental Material).

Canonical Correlation Analysis: We investigated the progression of PiB topography in CNnp, CNnn, and CNpp groups separately. To do this, we calculated the canonical correlation between the PiB topography at baseline and the PiB topography at follow-up using 42 ROIs (Hotelling, 1936, Hardle and Simar, 2007). A canonical correlation is the weighted average of variables (termed canonical variables) in one distribution that are maximally correlated with the weighted average of variables from another distribution. There can be multiple significant canonical correlations that isolate unique variance (similar to PCA components).

Let $X_1, X_2 \in \mathbb{R}^{N \times M}$ index the regional PiB SUVR in N subjects and M=42 ROIs at time-point 1 and 2, respectively. Define:

$$\Sigma = \begin{bmatrix} \Sigma_1 & \Sigma_{12} \\ \Sigma_{21} & \Sigma_2 \end{bmatrix} = \begin{bmatrix} X_1^T X_1 & X_1^T X_2 \\ X_2^T X_1 & X_2^T X_2 \end{bmatrix}$$

In this case, Σ is rank deficient which precludes matrix inversion. Therefore, define $\hat{\Sigma} = (1-\alpha)\Sigma + \alpha\Delta$ where α is an arbitrary parameter and Δ is a shrinkage target (Schafer and Strimmer, 2005). For this analysis, α is calculated in closed form as previously described (Ledoit and Wolf, 2003); Δ is defined as the diagonal matrix of Σ which ensures that $\hat{\Sigma}$ is full rank (Ledoit and Wolf, 2004). This approach was recently applied to analyze functional MRI data (Brier et al., 2015).

The number of significant canonical correlations is defined as the dimensionality of $\hat{\Sigma}_{12}$. Here we estimate this dimensionality using an information criterion (Minka, 2000). For every significant canonical correlation there exist two canonical variables: one corresponding to the baseline PiB topography and another corresponding to the follow up PiB topography. Let a_i be the ith canonical variable corresponding to the baseline PiB topography; a_i is defined as the ith eigenvector of $\hat{\Sigma}_1^{-1}\hat{\Sigma}_{12}\hat{\Sigma}_2^{-1}\hat{\Sigma}_{21}$. Similarly, let b_i be the ith canonical variable corresponding to the follow up PiB topography; b_i is defined as the ith eigenvector of $\hat{\Sigma}_2^{-1}\hat{\Sigma}_{21}\hat{\Sigma}_1^{-1}\hat{\Sigma}_{12}$. The values of a_i and b_i are unit norm and maximize the correlation between $a_i^T X_1$ and $b_i^T X_2$.

Penalized Regression: We fit an elastic net penalized regression model which uses the linear combination of an L_1 and L_2 norm of the calculated β values as a penalty (also known as Least Absolute Shrinkage and Selection Operator [LASSO] and ridge regression, respectively) (Tibshirani, 1996, Hastie et al., 2001, Zou and Hastie, 2005). Penalized regression differs from Ordinary Least Squares (OLS) regression in that it enforces a penalty term that forces some favorable property on the resulting regression βs. The L_1 penalty (LASSO) penalizes non-zero β values and thus forces small β values to 0 and retains a small number of non-zero β values. This results in a model that is more easily interpretable (i.e., has only a few terms to consider). However, in data that are highly co-

linear the decision to retain one variable and discard a highly correlated variable is arbitrary (Zou and Hastie, 2005). The elastic net accommodates this data feature by allowing highly correlated predictor variables to enter the model simultaneously. This flexibility is accomplished by relaxing the L_1 penalty with some fraction of an L_2 penalty.

We fit two separate elastic net models: The first uses follow-up MC SUVR as an outcome variable and the second uses percent change in MC SUVR as an outcome variable. Let $\boldsymbol{y} = [y_1, y_2, \dots, y_N] \in \mathbb{R}$ be the outcome variable of interest, either follow-up MC SUVR or the percent change in MC SUVR between baseline and follow-up. Further, let $X = \mathbb{R}^{N\times(M+1)}$ index the regional PiB SUVR in *N* subjects and *M*=40 ROIs. The M+1th region is the baseline MC-SUVR value which is included in order to investigate its sufficiency as a predictor. All variables were mean centered and made unit variance (z-scored). The estimate of penalized regression coefficients then has the form:

$$\underset{\hat{\beta}}{\operatorname{argmin}} \left\|\boldsymbol{y} - X\hat{\beta}\right\|^2 + \lambda\left((1-\alpha)\left\|\hat{\beta}\right\|^2 + \alpha\left\|\hat{\beta}\right\|_1\right)$$

The first term is OLS regression. The second and third terms are the L_2 and L_1 norms, respectively. λ determines the overall penalty severity and α determines the relative contribution of the L_1 and L_2 penalty. Both parameters are selected by leave-one-out cross validation.

To compare the power of baseline MC -SUVR and the entire topography to predict follow-up MC-SUVR or percentage change in MC -SUVR we compared the adjusted-R^2 values:

$$adj\ R^2 = 1 - \frac{(1-R^2)(N-1)}{N-df-1}$$

For OLS, the *df* is the number of predictors. Similarly, for LASSO regression the number of *df* is the number of non-zero β values. However, in elastic net regression the number of *df* is complicated by the potential for co-linearity in the selected predictors. Thus, *df* is defined as:

$$df = Tr\left(X_{\mathcal{A}}\left(X_{\mathcal{A}}^{T}X_{\mathcal{A}} + \left(\lambda \cdot (1-\alpha)\right)I\right)^{-1}X_{\mathcal{A}}^{T}\right)$$

where Tr indicates the trace and $\mathcal{A}$ indicates the active predictor set (Zou and Hastie, 2005).

Results

We first investigated the evolution of PiB topography in the CNnp group. The number of significant canonical correlations was determined to be 3 using an information criteria (Minka, 2000). The first canonical correlation was characterized by the baseline topography in Figure 1A and the follow-up topography in Figure 1B. The baseline topography was dominated by large positive weights in the posterior cingulate, precuneus, and superior temporal regions balanced by negative weights in lateral frontal regions. At follow-up the topography was similar but now also included inferior and lateral temporal regions. These topographies were highly correlated (Figure 1C; r = 0.58, $p < 10^{-4}$). For example, the precuneus and temporal regions are positive in both topographies and lateral frontal regions are strongly negative. The original regional data are then projected onto these topographies yielding a single scalar value for each subject at baseline and follow-up. Put another way, the weighted average of PiB binding (weighted according to the loadings in the topographies) was calculated at baseline and follow-up. These baseline and follow-up scalar values were highly correlated (Figure 1D; r = 0.99; $p < 10^{-10}$) with follow-up significantly higher. Thus, this canonical correlation represents the accumulation of Aβ locally in regions already affected.

The second canonical correlation, isolated after removing the variance related to the first canonical correlation, had a different pattern. The topographies of PiB for the baseline and follow-up scan are shown in Figure 1E and Figure 1F, respectively. The baseline topography was dominated by large positive weights in posterior cingulate, precuneus, and lateral parietal regions. In contrast, frontal regions dominated the follow-up topography. In contrast to the first canonical variable, the topographies were not as strongly correlated (Figure 1G; r = 031; $p = 0.04$). This indicates that baseline PiB topography predicts PiB

values in a different topography at follow-up. Projection of the original data onto these topographies resulted in a strong positive correlation (Figure 1H; r = 0.99; $p < 10^{-10}$). The prediction of a distinct topography at follow up based on a different topography at baseline suggests that this canonical correlation represents the expansion of Aβ topography to additional regions in the CNnp group.

The third canonical correlation has yet a different pattern. The topographies of PiB for the baseline and follow-up scan are shown in Figure 1I and Figure 1J and were moderately correlated (Figure 1K; r = 0.47, $p = 0.002$). The baseline topography was dominated by the anterior cingulate while the follow up topography was dominated by precuneus and subcortical regions. Projection of the original data onto this topography resulted in a strong positive correlation (Figure 1L; r = 0.99; $p < 10^{-10}$). This canonical correlation represents a combination of local and distributed processes distinct from the two aforementioned processes. These canonical correlation analyses were replicated using an alternative cut-off (Supplemental Material).

One analytic decision present in the previous results is the averaging of homotopic regions into a single bilateral ROI. We sought to determine whether this assumption (similar results on the left and right) was supported by the data. To accomplish this, the number of columns in X was doubled to $2M$ corresponding to left and right regional SUVR values being represented separately. The number of canonical correlations in this lateralized data set was determined to be 3 by an information criteria (Minka, 2000). This was the same number of canonical correlations identified in the homotopic analysis described above. There were two critical analytic questions to be addressed: 1) were the topographies isolated in the lateralized analysis symmetric across the mid-sagittal plane and 2) were the same topographies isolated in the lateralized analysis similar to those in the homotopic analysis. To address the first question, within a single topography the

loadings on the left were regressed onto the loadings on the right. For all topographies (3 canonical correlations with a baseline and follow-up topography) the left and right loadings were strongly related (all $p < 0.05$). In each case, the 95% confidence intervals on the regression β crossed 1, suggesting equal values for the left and the right hemispheres. Nevertheless, the maximum likelihood β estimate favored a larger representation in the left hemisphere compared to the right hemisphere but this bias was not significant. We next examined whether the topographies isolated in the lateralized analysis were similar to the topographies isolated in the homotopic analysis. For each canonical correlation (both baseline and follow-up), the topography resulting from the homotopic analysis was highly correlated between the left and right (analyzed separately) hemisphere topographies resulting from the lateralized analysis (all $r > 0.65$; all $p < 0.001$). These data suggest a bias towards representation of the left hemisphere but this difference does not lead to significantly different PiB accumulation with respect to hemisphere.

A single canonical correlation described the relationship between baseline and follow-up PiB topographies in the CNnn group (Figure 2A and B). The baseline and follow-up topographies were not correlated (Figure 2C) and did not demonstrate any obvious biological topography. Furthermore, the projected PiB values did not show systematic increases (Figure 2D), indicating no accumulation, consistent with their CNnn status.

A single canonical correlation described the relationship between baseline and follow-up PiB topographies in the CNpp group (Figure 3). The baseline topography (Figure 3A) was not significantly correlated with the follow-up topography (Figure 3B; $r = 0.12$, $p = 0.44$). However, the projected PiB values were strongly correlated ($r = 0.99$; $p < 10^{-10}$) and a dramatic accumulation was seen at follow-up, as reflected by data above the identity line (Figure 3D). These results suggest that CNpp individual at baseline continues to have significant accumulation longitudinally.

We next turned to the question of defining the correlates of future PiB accumulation in those who were PiB– at baseline. Accepting that the MC SUVR is a reliable measure of the level of AD pathology, can follow-up values be understood as a function of the baseline scan? Across all subjects, the baseline and follow-up MC SUVR were highly correlated ($r=0.55$, $p < 10^{-11}$, Adj-$R^2=0.30$) suggesting that, relative to the inter-individual variance, MC-SUVR values did not dramatically change over a period of 3 years. Importantly, only within just the CNnp group were the baseline and follow-up MC SUVR not correlated ($r=0.24$, $p=0.36$). Baseline MC-SUVR was also not correlated with the percent change in MC-SUVR across scans ($r=0.026$, $p=0.77$, Adj-$R^2=-0.007$). A negative Adj-R^2 indicates poor model fit. Notably, the baseline MC-SUVR and percent change in MC SUVR was negatively correlated in the CNpp group ($r=-0.54$, $p=0.0047$), suggesting a slowing of PiB accumulation within this topography. Thus, an open question is what features in the baseline topography correlates with future change in MC-SUVR.

To investigate which features of the baseline topography correlate with the follow-up MC-SUVR and percent change in MC-SUVR we fit two separate elastic net models using individual regional SUVR values as predictors. The β coefficients corresponding to the minimum cross-validation error for each model are shown in Table 2 and depicted visually in Figure 4. Positive values predict relatively higher MC-SUVR or positive changes in MC SUVR in the first (predicting MC-SUVR) and second model (predicting percent change in MC-SUVR) and negative values predict relatively lower MC-SUVR or negative changes in MC- SUVR. Importantly, the baseline MC-SUVR only entered the model for predicting follow-up MC-SUVR and not for predicting the change in MC-SUVR. The correlation between the predicted follow-up MC-SUVR and the actual MC-SUVR was significantly correlated ($r=0.73$, $p<10^{-22}$) and after correcting for the number of predictors was better than baseline MC SUVR alone (Adj-$R^2=0.50$ compared to 0.30). Similarly, the predicted percent change in MC-

SUVR was significantly correlated with the actual percent change in MC-SUVR ($r=0.54$, $p<10^{-10}$) and, after correcting for the number of predictors, was better than baseline MC-SUVR alone (Adj-$R^2=0.25$ compared to -0.007).

Discussion

This report uses two multivariate techniques (canonical correlation and penalized regression) to describe expanding Aβ topographies, as assessed by PiB, and identifies patterns of deposition correlated with future Aβ accumulation. Canonical correlation finds topographies that represent local and distributed accumulation in those who develop preclinical AD as assessed by a scalar cut-off. Critically, within cognitively normal individuals similar accumulation is absent in the group that remains PiB– at both time points but is present in those who became PiB+ at follow up or who remained PiB+ at both time points. Evidence of Aβ pathology (e.g., in the CNnp group) is sufficient for classification as Stage 1 preclinical AD (Sperling et al., 2011, Jack et al., 2012). Given that these participants became Aβ+ over a three year interval, this group represents the very earliest identifiable stages of preclinical AD. One method of operationalizing these criteria is through a scalar cut off applied to some mean Aβ binding index (Mintun et al., 2006). This is a potentially clinically useful method for summarizing the data and is predictive of eventual symptomatic conversion (Morris et al., 2009). However, the baseline MC SUVR failed to strongly correlate with future changes in MC SUVR, limiting its' utility in early disease stages. In contrast, the multivariate topography, not restricted to regions typically involved in the late stages of AD, strongly correlated with change in MC SUVR in a penalized regression analysis. Overall, these data demonstrate that accounting for the multivariate Aβ topography may help to understand and characterize the development of AD pathology over the different stages of the disease.

The application of multivariate statistics to the study of Aβ topographies allows for the investigation of novel neurobiology. Previous work employing mass univariate approaches necessarily included a data reduction step, e.g., averaging over many regions of interest. This approach is powerful when the relevant topography is known. However, in

the earliest stages of preclinical AD the relevant topography is unknown. Canonical correlation and penalized regression consider the entire topography in a minimally biased manner in order to maximize explanatory power. In this report, the additional power resulting from considering the entire distribution is leveraged to describe the topography of Aβ associated with conversion to preclinical AD. Importantly, the identified topography is distinct from the maximally effected topography in late stage AD.

A critical analytic decision in the analysis of PiB data is resolution at which the analysis should be conducted. Currently, three analytic strategies dominate the literature: analysis of 1) global PiB binding over the entire cortex, 2) composite PiB binding in an *a priori* topography, or 3) regional/voxel-wise PiB binding. The first two approaches are strong data reduction approaches that have demonstrated utility for predicting future symptomatic conversion (Morris and Price, 2001, Ma et al., 2014) though regional analysis may offer diagnostic advantages (Aizenstein et al., 2008). The notable feature of both of these approaches is the definition of a region of interest *a priori* (the whole cortex in the case of the former, specific topographies in the case of the latter). However, the relevant topography where Aβ deposition maximally occurs changes with disease progression (Villain et al., 2012); the topography that predicts symptomatic conversion may not be useful for detecting changes associated with earliest disease onset. Regional analyses allow for the flexibility to identify novel topographies but can yield varied results owing to the increased number of degrees-of-freedom (Engler et al., 2006, Jack et al., 2009, Grimmer et al., 2010, Rinne et al., 2010, Villemagne et al., 2011, Kadir et al., 2012). The presently reported results make use of multivariate techniques well-suited to compiling regional data into meaningful topographies. Application of such approaches is especially important when the topographies of interest are not known *a priori*.

The conversion from Aβ – to Aβ + status necessarily involves deposition of additional Aβ. That additional deposition is not haphazard but usually follows a stereotypical progression (Braak and Braak, 1997, Thal et al., 2002). We report on distinct processes using canonical correlation analysis. We find evidence of a local and distributed process. In the local process, regions with Aβ deposition at baseline simply accumulate more Aβ plaques by the follow up scan. However, in the distributed process, Aβ in a particular topography correlates with Aβ deposition in a different topography. Specifically, this progression represents the well-documented spread of Aβ deposition from posterior regions to more anterior regions (Thal et al., 2002).

The penalized regression model identifies topography of Aβ retention that correlates with future Aβ accumulation, either total value or percent change. Critically, the summary scalar was not a strong correlate of future percent change in Aβ burden. The regions with positive β values largely overlap with known areas of Aβ retention early in the disease (Thal et al., 2002). However, not all regions overlap with previously defined summary metrics used for operationalizing the Aβ + definition. Notably, the previous scalar metric was defined based on the observations in the symptomatic stage of AD and does not necessary reflect the spatial pattern of Aβ deposition in early preclinical stage (Mintun et al., 2006). This may lead to a biased view of early Aβ burden using the MC SUVR approach. The caudate nucleus deserves specific discussion. While it is known that caudate develops Aβ deposits throughout the disease (Thal et al., 2002), it is not commonly noted as a prominent initial contributor to Aβ topography in late onset AD (but see (Kemppainen et al., 2007)). Nevertheless, the caudate nucleus is identified in both penalized regression models. However, it has been noted that individuals with autosomal dominant AD have particularly intense Aβ retention in the striatum (Klunk et al., 2007). The independent evidence from

this study and the study of autosomal dominant AD suggests some important, or at least reliable, process may be ongoing in the striatum early in AD.

The penalized regression identified several regions which were negatively loaded in both regression models. The presence of these negatively loaded regions demonstrates that the Aβ topography associated with advancing disease is specific. That is, Aβ binding alone is not sufficient but rather it must be deposited in the disease causing topography. Amyloid in negatively loaded regions does not contribute to early disease pathophysiology. Indeed, Aβ is only present in the primary motor and sensory cortices much later in the disease (Braak and Braak, 1997), likely after symptoms develop. This potentially reflects that participants who have Aβ retention in the precentral gyrus are unlikely to be Aβ – at baseline. Thus, negatively loaded regions may serve as control regions by accounting for binding that is not due to Aβ or AD-related processes, especially at early disease stages. The presence of a negatively predictive region indicates that it is not simply high values of Aβ retention that predict conversion to Aβ + status, but rather Aβ deposition in a specific topography.

Only a small number of participants converted from Aβ – status to Aβ + status which limits the robustness of this study. Additionally, the definition of converter compared to non-converter is based on crossing an Aβ threshold. While such thresholds have been demonstrated to be potentially clinically useful (Mintun et al., 2006, Morris et al., 2009), other choices for a cutoff may be equally valid. Regardless of the precise definition, the primary scientific points remain: local and distributed processes are involved in the conversion to preclinical AD and consideration of the entire multivariate Aβ topography identifies correlates of advancing Aβ accumulation. A related point pertains to the prognostic information related to individuals who remain Aβ– for the duration of the study but accumulate more Aβ than average. These individuals are likely at an increased risk for developing preclinical AD in the future but longer follow-up time is required.

In summary, this study identified canonical correlations between baseline and follow-up PiB topographies in patients who converted from Aβ – to Aβ +. Penalized regression identified a topography association with future Aβ accumulation. Future studies could validate these regression models for their predictive power.

References

Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, Ziolko SK, James JA, Snitz BE, Houck PR, Bi W, Cohen AD, Lopresti BJ, DeKosky ST, Halligan EM, Klunk WE (2008) Frequent amyloid deposition without significant cognitive impairment among the elderly. Archives of neurology 65:1509-1517.

Braak H, Braak E (1997) Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiology of Aging 18:351-357.

Braak H, Del Tredici K (2012) Where, when, and in what form does sporadic Alzheimer's disease begin? Current opinion in neurology 25:708-714.

Braak H, Thal DR, Ghebremedhin E, Tredici KD (2011) Stages of the pathological process in Alzheimer disease: Age categories from 1 to 100 years. J Neuropathol Exp Neurol 70:960-969.

Brier MR, Mitra A, McCarthy JE, Ances BM, Snyder AZ (2015) Partial covariance based functional connectivity computation using Ledoit-Wolf covariance regularization. NeuroImage.

Engler H, Forsberg A, Almkvist O, Blomquist G, Larsson E, Savitcheva I, Wall A, Ringheim A, Langstrom B, Nordberg A (2006) Two-year follow-up of amyloid deposition in patients with Alzheimer's disease. Brain : a journal of neurology 129:2856-2866.

Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM (2002) Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33:341-355.

Grimmer T, Tholen S, Yousefi BH, Alexopoulos P, Forschler A, Forstl H, Henriksen G, Klunk WE, Mathis CA, Perneczky R, Sorg C, Kurz A, Drzezga A (2010) Progression of cerebral amyloid load is associated with the apolipoprotein E epsilon4 genotype in Alzheimer's disease. Biol Psychiatry 68:879-884.

Hardle W, Simar L (2007) Applied multivariate statistical analysis: Springer.

Hastie T, Tibshirani R, Friedman JH (2001) The elements of statistical learning : data mining, inference, and prediction : with 200 full-color illustrations. New York: Springer.

Hotelling H (1936) Relations between two sets of variates. Biometrika 28:321-377.

Jack CR, Jr., Knopman DS, Weigand SD, Wiste HJ, Vemuri P, Lowe V, Kantarci K, Gunter JL, Senjem ML, Ivnik RJ, Roberts RO, Rocca WA, Boeve BF, Petersen RC (2012) An operational approach to National Institute on Aging-Alzheimer's Association criteria for preclinical Alzheimer disease. Annals of neurology 71:765-775.

Jack CR, Jr., Lowe VJ, Weigand SD, Wiste HJ, Senjem ML, Knopman DS, Shiung MM, Gunter JL, Boeve BF, Kemp BJ, Weiner M, Petersen RC, Alzheimer's Disease Neuroimaging I (2009) Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. Brain : a journal of neurology 132:1355-1365.

Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, Shaw LM, Vemuri P, Wiste HJ, Weigand SD, Lesnick TG, Pankratz VS, Donohue MC, Trojanowski JQ (2013) Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. The Lancet Neurology 12:207-216.

Kadir A, Almkvist O, Forsberg A, Wall A, Engler H, Langstrom B, Nordberg A (2012) Dynamic changes in PET amyloid and FDG imaging at different stages of Alzheimer's disease. Neurobiol Aging 33:198 e191-114.

Kemppainen NM, Aalto S, Wilson IA, Nagren K, Helin S, Bruck A, Oikonen V, Kailajarvi M, Scheinin M, Viitanen M, Parkkola R, Rinne JO (2007) PET amyloid ligand [11C]PIB uptake is increased in mild cognitive impairment. Neurology 68:1603-1606.

Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Annals of neurology 55:306-319.

Klunk WE, Price JC, Mathis CA, Tsopelas ND, Lopresti BJ, Ziolko SK, Bi W, Hoge JA, Cohen AD, Ikonomovic MD, Saxton JA, Snitz BE, Pollen DA, Moonis M, Lippa CF, Swearer JM, Johnson KA, Rentz DM, Fischman AJ, Aizenstein HJ, DeKosky ST (2007) Amyloid deposition begins in the striatum of presenilin-1 mutation carriers from two unrelated pedigrees. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:6174-6184.

Ledoit O, Wolf M (2003) Improved estimation of the covariance matrix of stock returns with an application to portfolio selection. Journal of Empirical Finance 10:603-621.

Ledoit O, Wolf M (2004) A well-conditioned estimator for large-dimensional covariance matrices. Journal of Multivariate Analysis 88:365-411.

Ma Y, Zhang S, Li J, Zheng DM, Guo Y, Feng J, Ren WD (2014) Predictive accuracy of amyloid imaging for progression from mild cognitive impairment to Alzheimer disease with different lengths of follow-up: a meta-analysis. [Corrected]. Medicine (Baltimore) 93:e150.

Minka T (2000) Automatic choice of dimensionality for PCA. NIPS 13:598-604.

Mintun MA, Larossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, Klunk WE, Mathis CA, DeKosky ST, Morris JC (2006) [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. Neurology 67:446-452.

Morris JC (1993) The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 43:2412-2414.

Morris JC, Blennow K, Froelich L, Nordberg A, Soininen H, Waldemar G, Wahlund LO, Dubois B (2014) Harmonized diagnostic criteria for Alzheimer's disease: recommendations. Journal of internal medicine 275:204-213.

Morris JC, Price JL (2001) Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. Journal of molecular neuroscience : MN 17:101-118.

Morris JC, Roe CM, Grant E, Head D, Storandt M, Goate A, Fagan A, Holtzman DM, Mintun M (2009) Pitssburch compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. Archives of neurology 66:1469-1475.

Morris JC, Roe CM, Xiong C, Fagan AM, Goate AM, Holtzman DM, Mintun MA (2010) APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. Annals of neurology 67:122-131.

Price JL, McKeel DW, Jr., Buckles VD, Roe CM, Xiong C, Grundman M, Hansen LA, Petersen RC, Parisi JE, Dickson DW, Smith CD, Davis DG, Schmitt FA, Markesbery WR, Kaye J, Kurlan R, Hulette C, Kurland BF, Higdon R, Kukull W, Morris JC (2009) Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease. Neurobiol Aging 30:1026-1036.

Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE, Mathis CA, Blennow K, Barakos J, Okello AA, Rodriguez Martinez de Liano S, Liu E, Koller M, Gregg KM, Schenk D, Black R, Grundman M (2010) 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with

bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. Lancet neurology 9:363-372.
Rousset OG, Ma Y, Evans AC (1998) Correction for partial volume effects in PET: principle and validation. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 39:904-911.
Schafer J, Strimmer K (2005) A shrinkage approach to large-scale covariance matrix estimation and implications for functional genomics. Statistical applications in genetics and molecular biology 4:Article32.
Sojkova J, Zhou Y, An Y, Kraut MA, Ferrucci L, Wong DF, Resnick SM (2011) Longitudinal patterns of beta-amyloid deposition in nondemented older adults. Arch Neurol 68:644-649.
Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr., Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association 7:280-292.
Su Y, Blazey TM, Snyder AZ, Raichle ME, Marcus DS, Ances BM, Bateman RJ, Cairns NJ, Aldea P, Cash L, Christensen JJ, Friedrichsen K, Hornbeck RC, Farrar AM, Owen CJ, Mayeux R, Brickman AM, Klunk W, Price JC, Thompson PM, Ghetti B, Saykin AJ, Sperling RA, Johnson KA, Schofield PR, Buckles V, Morris JC, Benzinger TL (2014) Partial Volume Correction in Quantitative Amyloid Imaging. Neuroimage.
Su Y, Blazey TM, Snyder AZ, Raichle ME, Marcus DS, Ances BM, Bateman RJ, Cairns NJ, Aldea P, Cash L, Christensen JJ, Friedrichsen K, Hornbeck RC, Farrar AM, Owen CJ, Mayeux R, Brickman AM, Klunk W, Price JC, Thompson PM, Ghetti B, Saykin AJ, Sperling RA, Johnson KA, Schofield PR, Buckles V, Morris JC, Benzinger TL, Dominantly Inherited Alzheimer N (2015) Partial volume correction in quantitative amyloid imaging. NeuroImage 107:55-64.
Su Y, D'Angelo GM, Vlassenko AG, Zhou G, Snyder AZ, Marcus DS, Blazey TM, Christensen JJ, Vora S, Morris JC, Mintun MA, Benzinger TL (2013) Quantitative analysis of PiB-PET with FreeSurfer ROIs. PloS one 8:e73377.
Thal DR, Rub U, Orantes M, Braak H (2002) Phases of A -deposition in the human brain and its relevance for the development of AD. Neurology 58:1791-1800.
Tibshirani R (1996) Regression shrinkage and selection via the Lasso. J Roy Stat Soc B Met 58:267-288.
Villain N, Chetelat G, Grassiot B, Bourgeat P, Jones G, Ellis KA, Ames D, Martins RN, Eustache F, Salvado O, Masters CL, Rowe CC, Villemagne VL, Group AR (2012) Regional dynamics of amyloid-beta deposition in healthy elderly, mild cognitive impairment and Alzheimer's disease: a voxelwise PiB-PET longitudinal study. Brain : a journal of neurology 135:2126-2139.
Villemagne VL, Pike KE, Chetelat G, Ellis KA, Mulligan RS, Bourgeat P, Ackermann U, Jones G, Szoeke C, Salvado O, Martins R, O'Keefe G, Mathis CA, Klunk WE, Ames D, Masters CL, Rowe CC (2011) Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. Annals of neurology 69:181-192.
Vlassenko AG, Mintun MA, Xiong C, Sheline YI, Goate AM, Benzinger TL, Morris JC (2011) Amyloid-beta plaque growth in cognitively normal adults: Longitudinal [(11) C]Pittsburgh compound B data. Ann Neurol 70:857-861.
Zou H, Hastie T (2005) Regularization and variable selection via the elastic net. J Roy Stat Soc B 67:301-320.

Tables

Table 1: Demographic Information

Variable	**CNnn**	**CNnp**	**CNpp**	***p***
N	115	16	26	N/A
Age in years at baseline scan (SD)	59.5 (8.77)	66.4 (8.67)	68.2 (6.3)	<0.001
Gender (M/F)	35/80	5/11	10/16	0.73
Education in years (SD)	16.1 (2.4)	15.4 (2.5)	15.4 (2.4)	0.90
APOE ε4+ (% Carriers)	23 (20%)	9 (56%)	17 (65%)	<0.001
MC SUVR at baseline (SD)	0.97 (0.07)	1.17 (0.10)	2.12 (0.60)	<0.001
MC SUVR (Follow-up)	1.02 (0.09)	1.47 (0.31)	2.48 (0.56)	<0.001
MMSE at baseline)	29.3 (0.86)	29.3 (1.09)	28.9 (1.51)	0.73
MMSE at follow-up)	29.4 (0.92)	29.4 (0.96)	29.0 (1.42)	0.89
Follow-up time in years (SD)	3.30 (1.45)	3.26 (1.20)	2.99 (1.26)	0.37

Table 1 Caption: Mean (standard deviation) or counts for demographic variables. CNnn, cognitively normal individuals, PiB-negative at both scans; CNnp, cognitively normal, PiB–negative at first scan but PiB-positive at second scan; M, males; F, females; APOE ε4, positive indicates at least one APOE ε4 allele; MC SUVR, mean cortical standard uptake volume ratio of PiB deposition MMSE, mini mental status exam SD, standard deviation

Table 2: Elastic Net Regression Coefficients

	Predictor Value	
Region	**Follow-up MC-SUVR**	**% Change in MC-SUVR**
Posterior Cingulate	0.032	0.023
Rostral Middle Frontal	0.028	
Rostral Anterior Cingulate	0.027	0.014
Precuneus	0.025	
Baseline MC-SUVR	0.021	
Caudate	0.017	0.011
Frontal Pole	0.016	0.002
Caudal Anterior Cingulate	0.010	0.004
Caudal Middle Frontal	0.005	
Parahippocampal Gyrus	0.003	
Pericalcerine		−0.003
Transverse Temporal	−0.001	
Insula	−0.004	−0.004
Pre-central Gyrus	−0.015	−0.016
Post-central Gyrus	−0.027	−0.023

Table 2: Table of penalized (elastic net) regression coefficients. Blank spaces correspond to coefficients equal to zero.

Figure Captions

Figure 1 Caption: *Canonical correlation analysis reveals distinct amyloid accumulation processes.* The results corresponding to the first, second, and third canonical correlations are organized as individual rows. The first column (A, E, I) shows the canonical variable (a) corresponding to the baseline PiB topography. The second column (B, F, J) shows the canonical variable (b) corresponding to the follow up PiB topography. These topographies are unit norm. The third column (C, G, K) shows the correlation between the two canonical variables within a single canonical correlation. The fourth column (D, H, L) show the projection of the original data onto the canonical variables. Red line is the identity line.

Figure 2 Caption: *Canonical correlation analysis in the CNnn group reveals minimal amyloid accumulation.* Canonical correlation analysis in the CNnn group using the same presentation style as Figure 1 in the main text. The critical feature here is that the correlated topographies do not exhibit marked accumulation (the data are along the identity line in D).

Figure 3 Caption: *Canonical correlation analysis in the CNpp group reveals substantial amyloid accumulation.* Canonical correlation analysis in the CNpp group using the same presentation style as Figure 1 in the main text. The critical feature here is that the correlated topographies exhibit marked accumulation (the data are above the identity line in D).

Figure 4 Caption: *Topography of baseline PiB deposition that correlates with follow-up and % change in MC-SUVR.* Graphical representation of the information in Table 2. First row shows MC definition. Color bar indicates regression β values.

Supplemental Figure 1 Caption: *Baseline and Follow-up SUVR Images for the CNnn, CNnp and CNpp Group.* Baseline and follow-up SUVR images for three randomly selected participants in each group.

Supplemental Figure 2 Caption: *Canonical Correlation Analysis of CNnp is Robust to Choice of Threshold.* A critical analytic step is the definition of PiB+ vs. PiB–. The canonical correlation analysis was repeated for two different MC-SUVR thresholds. In each case, 3 significant canonical correlations were isolated. The correlation between the baseline and follow-up topographies in each canonical correlation was assessed. (A) The correlation matrix between the three baseline and follow-up topographies under the two thresholds are shown. The critical finding is in the off-diagonal box representing the cross correlation between the two thresholds. For each of the three topographies using a 1.42 threshold are identifiable using an alternative 1.20 threshold (see Supplemental Materials). (B) The individual topographies for each threshold are shown. The visual correspondence confirms the quantitative information in A.