Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix

eMethods 1: ADNI cohort

The primary goal of ADNI has been to test whether serial MRI, 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 coinvestigators 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.

eMethods 2: CSF collection

CSF was collected into polypropylene collection tubes or syringes and transferred into polypropylene transfer tubes without any centrifugation step. CSF was thereafter frozen on dry ice within 1 hour after collection, and shipped overnight to the ADNI Biomarker Core on dry ice. The samples were thawed (1 hour) at room temperature, gently mixed and aliquoted (0.5 mL) into bar code–labeled polypropylene vials and stored at -80°C.

eMethods 3: PET images processing

The UU Center for Alzheimer's Care, Imaging and Research processed images using 3-dimensional stereotactic surface projections computed using Neurostat1. PET scans were downloaded from LONI using the postprocessed group 4 images, i.e. coregistered and averaged frames, standardized image orientation and voxel size, and uniform resolution smoothed to 8 mm. Neurostat aligned the brain images along the AC-PC line and nonlinearly warped the image into standard Talairach space. Longitudinal scans, for each subject, were coregistered to the baseline scan and a multistep nomalization was used to create a mean template. A peak pixel template was created from the mean template and applied to intrasubject serial scans to produce surface projection maps, SSPs. Neurostat predefined brain regions in Talairach space were used to calculate the ROI regional values based on the SSP maps. AV45 images were normalized using both cerebellar and white matter values. Neurostat automatically defined the averaged cerebellar value. White matter values were determined by sampling pixels from the amyloid image, in Talairach-space, starting at a much greater depth, thus bypassing the cortical ribbon. The Neurostat generated global value was used for the whole brain white matter value.

UC Berkeley used SPM5 software to coregister the AV-45 PET scans with the corresponding MRI scans, that previously were segmented and parcellated with Freesurfer (v 4.5) as described[2]. Fully preprocessed format (series description in LONI

Advanced Search: “AV45 Coreg, Avg, Std Img and Vox Siz, Uniform Resolution”) were downloaded. Each subject’s first florbetapir image was coregistered using SPM5 to that subject’s MRI image (series description: ADNI 1scans *N3;* and ADNI GO/2 scans *N3*) that was closest in time to the florbetapir scan. Freesurfer processing was carried out to skull-strip, segment, and delineate cortical and subcortical regions in all MRI scans. Florbetapir means from grey matter in subregions were extracted within 4 large regions (frontal, anterior/posterior cingulate, lateral parietal, lateral temporal)[3,4].

To account for the varying sizes of the subregions,weighted means for each of the 4 main regions (frontal, anterior/posterior cingulate, lateral parietal, lateral temporal) were created:

(subregion1_florbetapir_mean X subregion1_volume) + (subregion2_florbetapir_mean X subregion2_volume) + …. (subregionN_florbetapir_mean X subregionN_volume) / (subregion1_volume + subregion2_volume +… subregionN_volume)

Conventional (nonweighted) average of whole cerebellum was selected as reference for the summary CB measure. Means from three Freesurfer-defined reference regions (whole cerebellum, brainstem/pons and eroded subcortical white matter) were selected as reference for the summary composite measure.

eTable 1. Cutoffs obtained for the different Aβ measures using mixture models.

Aβ measures	Cutoff value
CSF $A\beta_{1-42}$	191 ng/mL
Average CB	1.26 SUVR
Average WM	0.73 SUVR
Summary CB	1.12 SUVR
Summary Composite	0.805 SUVR

eTable 2. Fitting of the different models in the training and test set.

Model	Average CB		Average WM		Summary CB		Summary Composite	
	Training	Test	Training	Test	Training	Test	Training	Test
Linear	0.55	0.48	0.64	0.62	0.59	0.52	0.61	0.61
Negative Exponential	0.56	0.54	0.66	0.64	0.62	0.59	0.69	0.66
Polynomial	0.57	0.54	0.69	0.64	0.62	0.59	0.68	0.65
Hyperbolic	0.62	0.56	0.68	0.64	0.64	0.60	0.70	0.65
MARS	0.67	0.59	0.70	0.66	0.66	0.61	0.70	0.66

eTable 3. SUVR corresponding to CSF $A\beta_{1-42}$ 192 pg/mL value for subjects with 1 *APOE* ε4 allele.

	SUVR for 0 copies *APOE* ε4 allele	SUVR for 1 copy *APOE* ε4 allele
Average CB	1.27	1.19
Average WM	0.72	0.69
Summary CB	1.13	1.09
Summary Composite	0.81	0.79

eTable 4. Clinical diagnosis of subjects with mismatch classification based on CSF $A\beta_{1-42}$ and summary cerebellum cutoffs.

	Abnormal CSF $A\beta_{1-42}$ Normal Summary cerebellum	Normal CSF $A\beta_{1-42}$ Abnormal Summary cerebellum
CN	28 (40.0%)	13 (48.0%)
MCI	38 (54.0%)	14 (52.0%)
AD	4 (6.0%)	0 (0.0%)

Number of cases (percentages in each group).

eTable 5. Differences in cognitive tests in subjects with mismatch classification based on CSF $A\beta_{1-42}$ and summary cerebellum cutoffs.

	Abnormal CSF $A\beta_{1-42}$ Normal Summary cerebellum	
	Coefficient (S.D.)	*P* value
Executive Domain	0.22 (0.09)	0.012
Memory Domain	-0.45 (0.13)	0.001
ADAS-cog	-0.86 (1.0)	0.39

eTable 6. Transformation values from the different Aβ PET pipelines. Summary CB was selected as reference and all the other pipelines were compared to cerebellum.

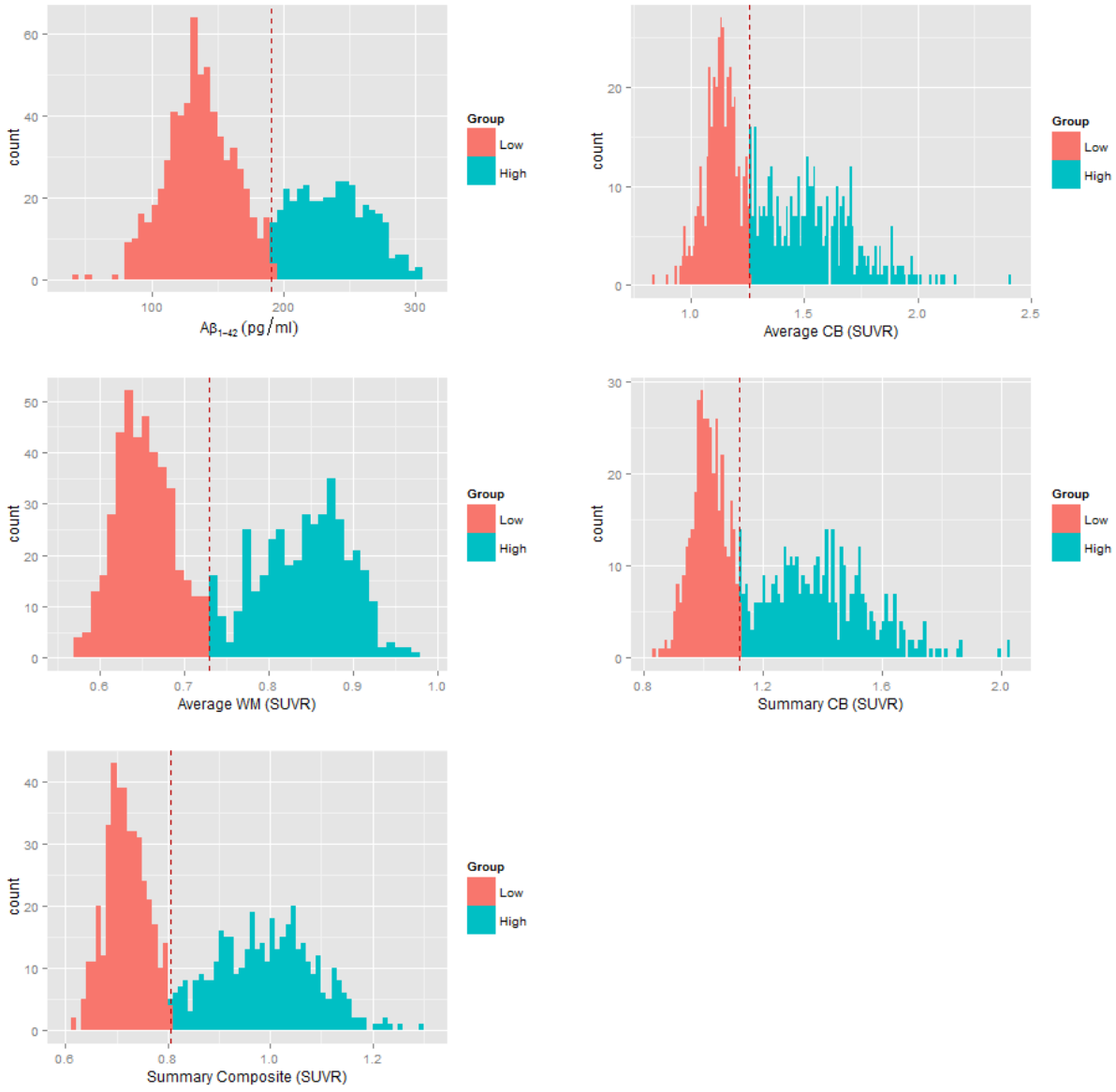
	Intercept	Slope
Average CB	0.04	0.88
Average WM	-0.19	1.89
Summary Composite	0.02	1.40

eTable 7. Association between ADAS-cog values and Aβ biomarkers stratified by diagnosis.

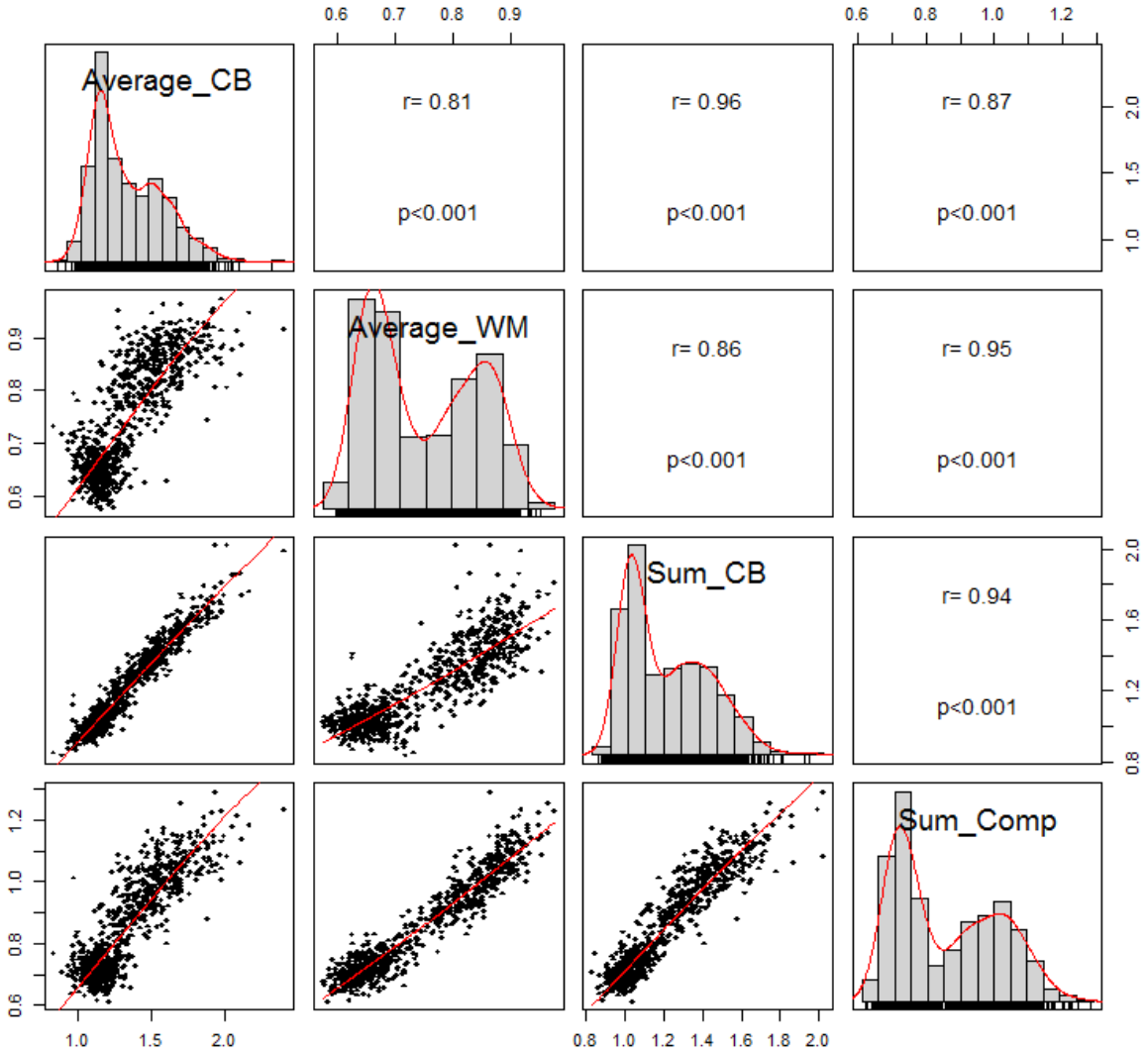
	CSF $A\beta_{1-42}$	Summary Composite
CN	0.09	0.09
MCI	0.22	0.27
AD	0.06	0.05

R^2 values.

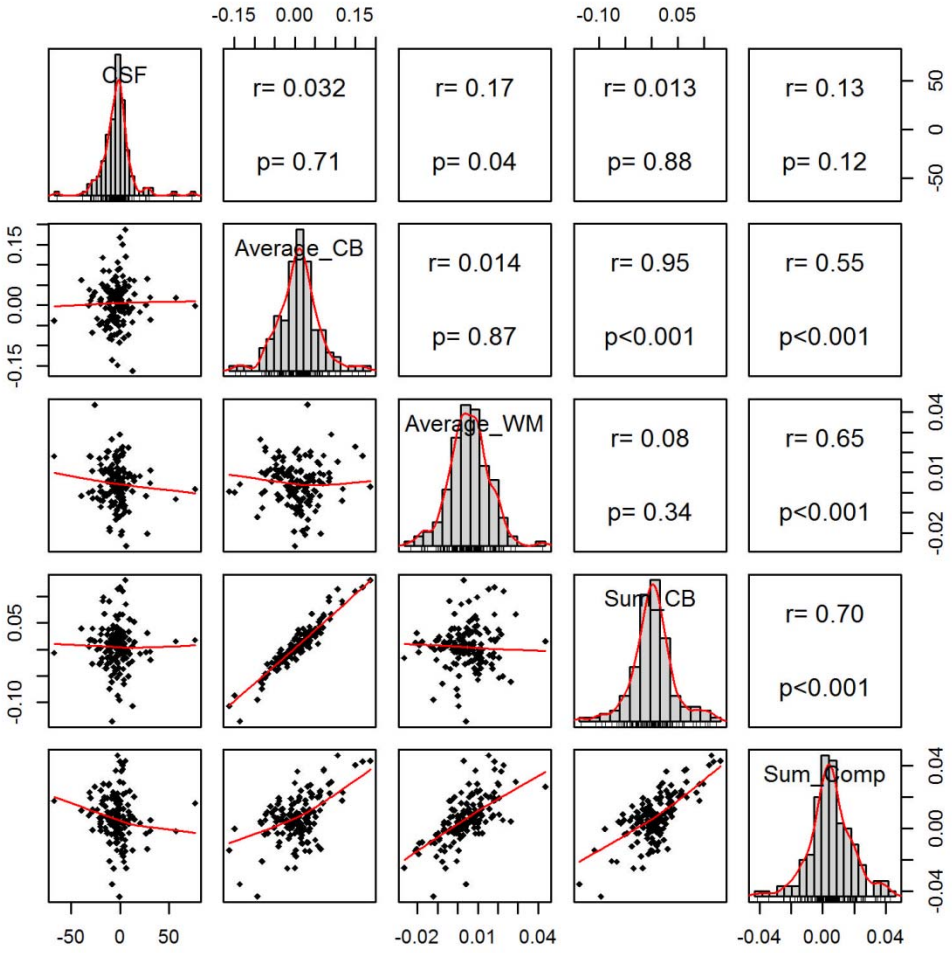
eFigure 1. Distribution of mixtures of baseline Aβ measures. All measures showed the presence of 2 groups except the average WM measure that showed the best result with 3 groups; nevertheless we selected the model with 2 groups to be consistent with other measures. Red dashed line represents cutoff value.



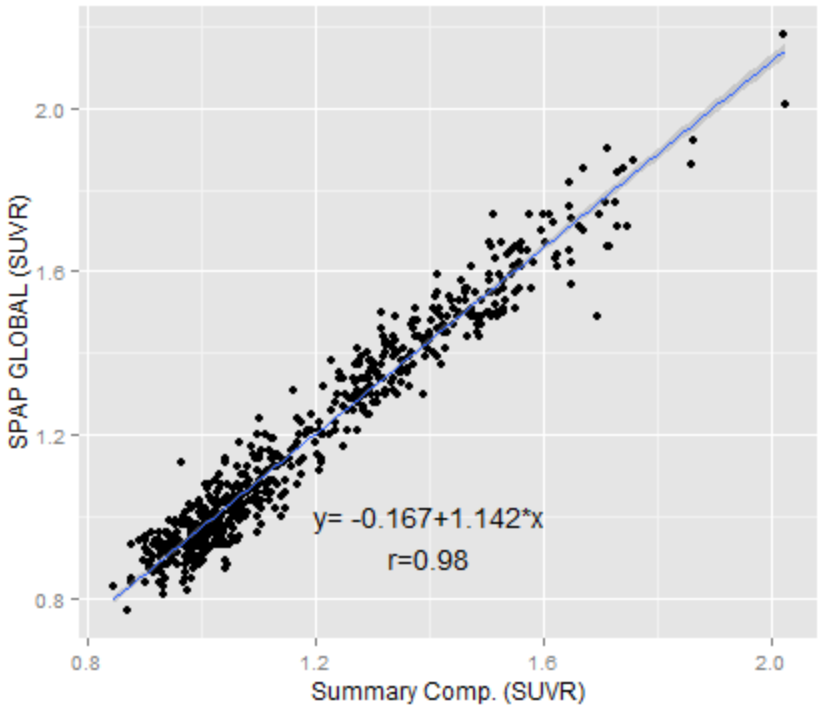
eFigure 2. Panel showing the individual scatterplots (below diagonal) and Pearson correlation coefficients (above the diagonal) for the different AV-45 PET measurements at the baseline visit (diagonal).



eFigure 3. Matrix showing the individual scatterplots depicting CSF $A\beta_{1-42}$ and PET Aβ SUVR changes during a 2-year follow-up (below the diagonal) and the corresponding Pearson correlation coefficients (above the diagonal).



eFigure 4. Correlation and transformation formula for the summary composite and global SPAP AV-45 PET measures.



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