Supplementary Online Content

Soldan A, Pettigrew C, Cai Q, et al; BIOCARD Research Team. Hypothetical preclinical Alzheimer disease groups and longitudinal cognitive change. *JAMA Neurol*. Published online April 11, 2016. doi:10.1001/jamaneurol.2016.0194.

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

eMethods 1. Selection of Participants

A total of 354 individuals were initially enrolled in the study. At enrollment, subjects in this study were admitted to the Clinical Center at the National Institutes of Health for 3 days. Subjects were enrolled over time, beginning in 1995 and ending in 2005. They received a detailed physical, neurological and psychiatric examination, an electrocardiogram, and standard laboratory studies (e.g., complete blood count, vitamin B12, thyroid function, etc.). A comprehensive neuropsychological battery was administered at baseline that included the following: the complete Wechsler Adult Intelligence Scale- revised, the complete Wechsler Memory Scale - Revised, a version of the Buschke Cued Selective Reminding test developed by the Geriatric Psychiatry Branch (GPB) of NIMH, the Stroop Test, the Delis-Kaplan Executive Function System test, Clock drawing, the Rey-Osterreith Complex Figure (copy and recall), the Boston Naming test (30-item version), Letter and Category Fluency, the National Adult Reading Test (NART), the Mattis Dementia Rating Scale, and the Mini-Mental State Examination (MMSE). Mood was assessed with the Hamilton Depression Scale, the Beck Depression Inventory and the Spielberger Anxiety Scale. A Clinical Dementia Rating (CDR) was generated by GPB staff. During the 3-day baseline visit, a magnetic resonance imaging (MRI) scan was obtained and a lumbar puncture was performed.

The staff of the GPB reviewed the results of the clinical and cognitive assessments and excluded participants who were judged to be cognitively impaired, as determined by the cognitive testing or by evidence of clinical symptoms based on reports by collateral sources. Subjects were also excluded who had a history of significant medical problems such as severe cardiovascular disease (e.g., atrial fibrillation), chronic psychiatric disorders (e.g., schizophrenia, alcohol or drug abuse), chronic neurologic disorders (e.g., epilepsy, multiple sclerosis) or severe cerebrovascular disease (based on the MRI scan). Five subjects did not meet the entry criteria and were excluded at baseline, leaving a total 349 participants who were followed over time.

eMethods 2. Reasons for Exclusion of Subjects From Analyses

Subjects were successively excluded from analyses for the following reasons: (1) the first CSF draw was insufficiently coincident with the cognitive assessments (i.e., occurred more than 6 months after the baseline cognitive and clinical data were obtained) (n=76); (2) subjects had not yet re-enrolled in the study or had withdrawn (n=31); (3) the estimated age of onset of clinical symptoms was determined to be at or prior to baseline, based on the report of the subject and an informant (n=6).

eMethods 3. Consensus Diagnoses

Each case included in these analyses received a consensus diagnosis by the staff of the BIOCARD Clinical Core at Johns Hopkins. This research team included: neurologists, neuropsychologists, research nurses and research assistants. During the study visit, each subject had received a comprehensive cognitive assessment and a Clinical Dementia Rating (CDR), as well as a comprehensive medical evaluation (including a medical, neurologic and psychiatric assessment). For the cases with evidence of clinical or cognitive dysfunction, a clinical summary was prepared that included information about demographics, family history of dementia, work history and past history of medical, psychiatric and neurologic disease, medication use and results from the neurologic and psychiatric evaluation. The reports of clinical symptoms from the subject and collateral sources were summarized, and the results of the neuropsychological testing were reviewed. The neuropsychological test scores that were used to calculate the cognitive composite outcome measure were part of the larger neuropsychological battery reviewed during the consensus diagnoses. See Albert et al., 2014 for the complete battery.[1] Thus, the diagnostic process for each case was handled in a similar manner: (1) clinical data were examined pertaining to the medical, neurologic and psychiatric status of the subject, (2) reports of changes in cognition by the subject and by collateral sources were examined, and (3) decline in cognitive performance was established. These data were used to: (1) determine whether the subject had become cognitively impaired, (2) determine the likely etiology of the impairment, if the subject was impaired, and (3) determine the age at which the clinical symptoms began. These diagnostic procedures are identical to those implemented by the 27 Alzheimer's Disease Centers in the US supported by the National Institute on Aging.

[1] Albert M, Soldan A, Gottesman R, et al. Cognitive changes preceding clinical symptom onset of mild cognitive impairment and relationship to ApoE genotype. Current Alzheimer research 2014;11:773-784.

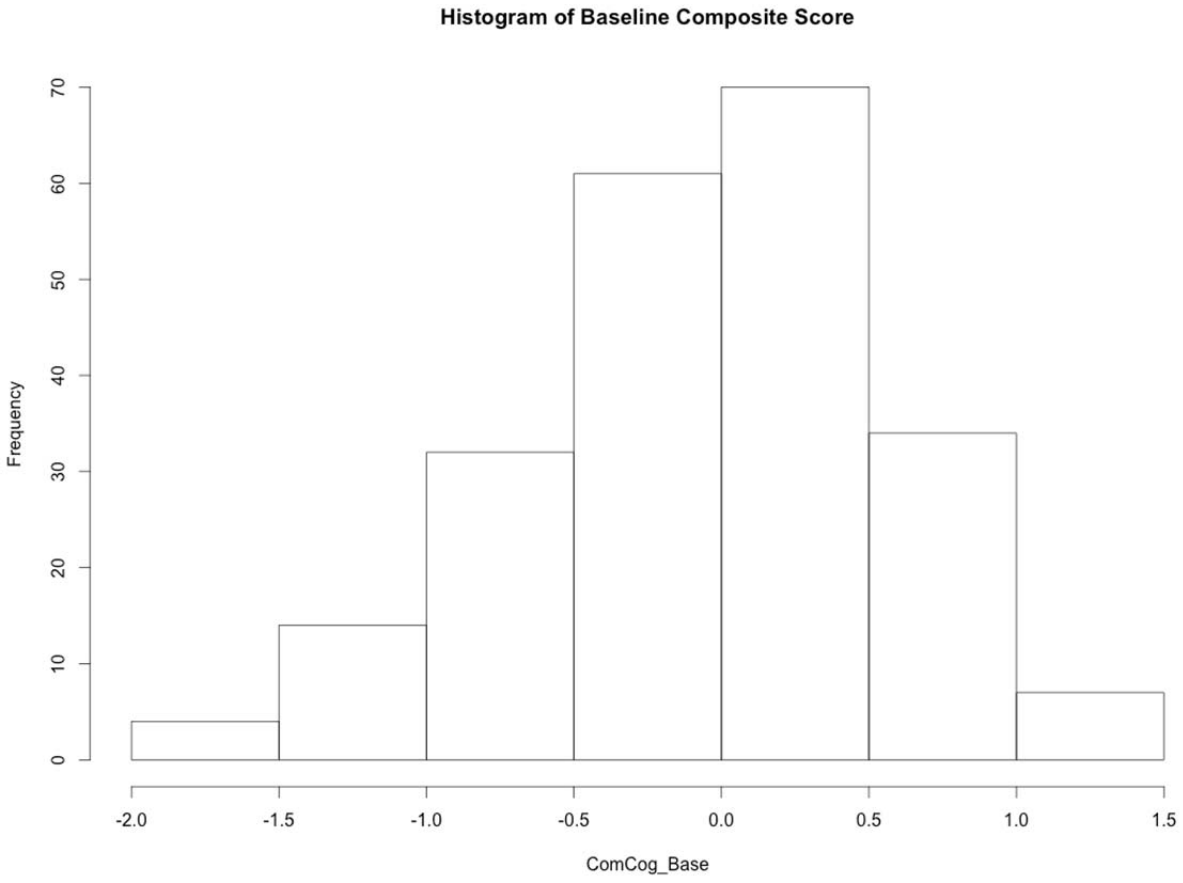
eMethods 4. CSF Assessments

The AlzBio3 kit contains monoclonal antibodies specific for Aβ1-42 (4D7A3), t-tau (AT120), and p-tau181p (AT270), each chemically bonded to unique sets of color-coded beads, and analyte-specific detector antibodies (HT7, 3D6). Calibration curves were produced for each biomarker using aqueous buffered solutions that contained the combination of three biomarkers at concentrations ranging from 25 to 1,555 pg/ml for recombinant tau, 54 to 1,799 pg/ml for synthetic Aβ1-42 peptide, and 15 to 258 pg/ml for a tau synthetic peptide phosphorylated at the threonine 181 position (i.e., the p-tau181p standard). Each subject had all samples (run in triplicate) analyzed on the same plate. The intra-assay coefficients of variation (CV) for plates used in this study were: 7.7% +/- 5.3 ($A\beta_{1-42}$); 7.1% +/- 4.9 (tau); 6.3% +/- 4.8 ($p\text{-}tau_{181}$). Interassay (plate-to-plate) CVs for a single CSF standard run on all plates used in this study were: 8.9% +/- 6.5 ($A\beta_{1-42}$); 4.7% +/-3.3 (tau); 4.3% +/- 3.18 ($p\text{-}tau_{181}$). Compared with studies using the same kits and platforms[1-2] our absolute results are at the median levels for tau, $p\text{-}tau_{181}$ and $A\beta_{1-42}$. The CVs, plate-to-plate variability, and the dynamic range of our assays are well within published norms[1, 2].

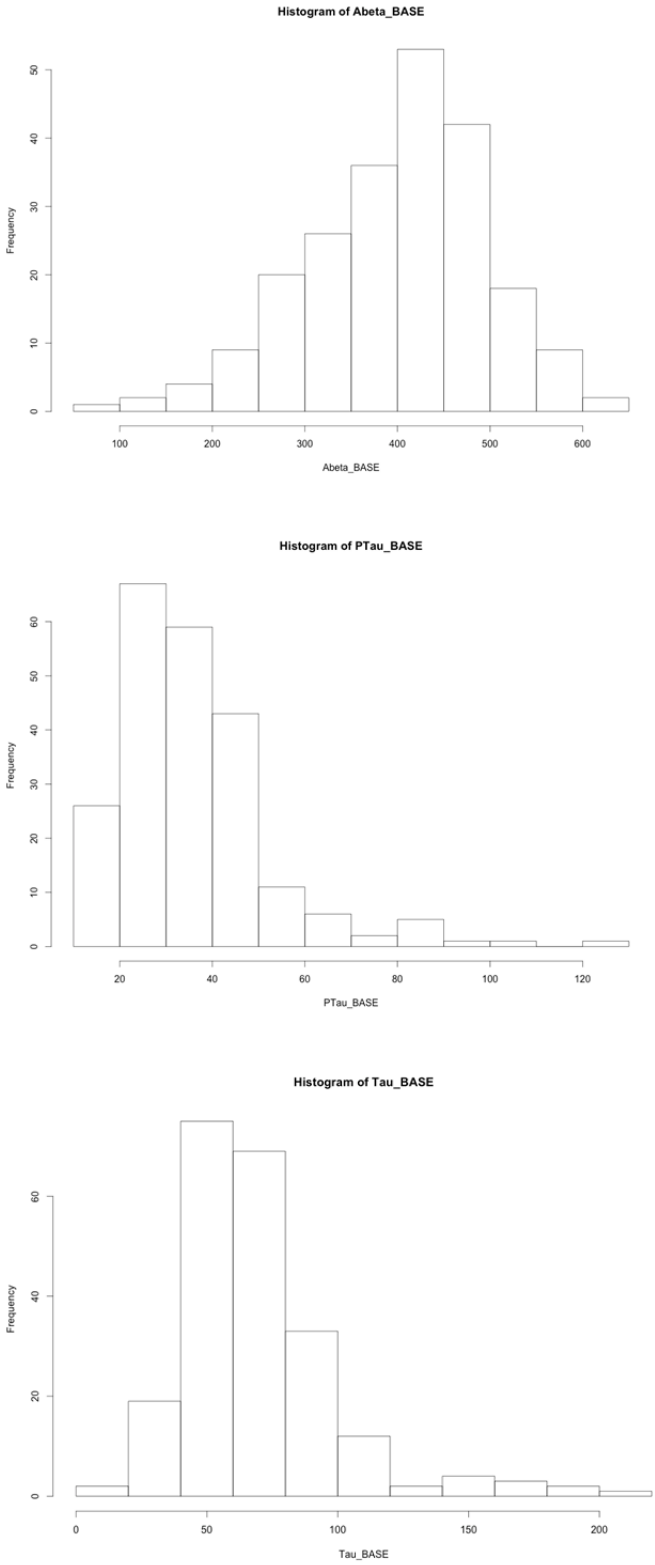
[1.] Shaw L, Vanderstichele H, Knapik—Czajika M, et al. Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid biomarker signature in Alzheimer's Disease Neuroimaging Initiative subjects. Ann Neuol 2009; 65: 403-413.

[2.] Mattson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. JAMA 2009; 302: 385-393.

eFigure 1. Frequency Distribution of Cognitive Composite Score at Baseline: for All Subjects in the Analyses (N=222)



eFigure 2. Frequency Distribution of CSF Biomarkers at Baseline: abeta $_{1\text{-}42}$ (Top), CSF p-tau (Middle), and CSF Total tau at Baseline for Subjects in the Analyses



eTable 1. Results of Linear Mixed Models Using Quintiles to Define the Hypothetical Preclinical AD Groups

	Groups defined by CSF abeta and p-tau			Groups defined by CSF abeta and total tau		
Model Predictors	Estimate	SE	p-value	Estimate	SE	p-value
Time	0.0889	0.0321	0.0062	0.0859	0.0326	0.0092
Baseline age	-0.0165	0.0042	0.0001	-0.0159	0.0043	0.0003
Gender (male)	-0.2523	0.0782	0.0015	-0.2526	0.0784	0.0015
Education	0.0477	0.0165	0.0044	0.0499	0.0164	0.0027
Stage 1	-0.0755	0.1193	0.5273	-0.0400	0.1212	0.7417
Stage 2	-0.2318	0.1362	0.0903	-0.2225	0.1352	0.1014
SNAP	-0.1269	0.1207	0.2940	-0.0236	0.1194	0.8436
Baseline age x time	-0.0019	0.0004	<0.0001	-0.0018	0.0004	<0.0001
Gender x time	-0.0028	0.0068	0.6852	-0.0035	0.0068	0.7778
Education x time	0.0017	0.0014	0.2436	0.0018	0.0015	0.2245
Stage 1 x time	-0.0040	0.0112	0.7200	-0.0184	0.0113	0.1061
Stage 2 x time	-0.0706	0.0136	<0.0001	-0.0540	0.0141	0.0002
SNAP x time	-0.0071	0.0105	0.5030	-0.0176	0.0104	0.0923

Note: abnormal levels of abeta were defined as being in the lower 1/5th (i.e. 20%) of the distribution and abnormal levels of tau/ p-tau were defined as being in the upper 1/5th of the distribution, which resulted in the following cut-off values: 319.1 pg/mL for $abeta_{1-42}$, 44.7 pg/mL for p-tau, and 83.7 pg/mL for tau. Stage 0 was used as the implicit baseline in these models. Thus, estimates for Stage 1, 2, and SNAP and their interactions with time reflect differences relative to Stage 0.

eTable 2. Number and Percentage of Individuals Who Progressed to MCI or AD Dementia: Among the 4 Hypothetical Preclinical AD Groups Using 3 Different Cut-Points to Define Group Membership

	Groups defined by CSF abeta and p-tau		Groups defined by CSF abeta and total tau	
Cut-point: tertiles[1]				
Baseline Group	Total N	Progressed N (%)	Total N	Progressed N (%)
Stage 0	102	18 (17.6%)	102	20 (19.6%)
Stage 1	46	9 (19.6%)	46	9 (19.6%)
Stage 2	28	15 (53.6%) **	28	15 (53.6%) **
SNAP	46	9 (19.6%)	46	7 (15.2%)
Cut-point: quintiles[2]				
Stage 0	151	27 (17.9%)	152	26 (17.1%)
Stage 1	26	6 (23.1%)	25	7 (28.0%)
Stage 2	19	12 (63.2%) **	20	11 (55.0%) **
SNAP	26	6 (23.1%)	25	7 (28.0%)
Cut-point: median[3]				
Stage 0	47	7 (14.9%)	47	9 (19.1%)
Stage 1	64	10 (15.6%)	64	12 (18.8%)
Stage 2	47	21 (44.7%) **	47	19 (40.4%) *
SNAP	64	13 (20.3%)	64	11 (17.2%)

[1] Abnormal abeta levels are defined as being in the lower 1/3 of the distribution; abnormal tau/ p-tau levels are defined as being in the upper 1/3 of distribution.
[2] Abnormal abeta levels are defined as being in the lower 1/5 of the distribution; abnormal tau/ p-tau levels are defined as being in the upper 1/5 of distribution.
[3] Abnormal abeta levels are defined as being in the lower 1/2 of the distribution; abnormal tau/ p-tau levels are defined as being in the upper 1/2 of distribution.
* Significantly greater proportion of individuals who progress compared to all other groups by chi-square test (all $p<0.05$).
** Significantly greater proportion of individuals who progress compared to all other groups by chi-square test (all $p<0.01$).