Comparing Positron Emission Tomography Imaging and Cerebrospinal Fluid Measurements of β-Amyloid

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Objective: We examined agreement and disagreement between 2 biomarkers of β-amyloid (Aβ) deposition (amyloid positron emission tomography [PET] and cerebrospinal fluid [CSF] $A\beta_{1-42}$) in normal aging and dementia in a large multicenter study.
Methods: Concurrently acquired florbetapir PET and CSF Aβ were measured in cognitively normal, mild cognitive impairment (MCI), and Alzheimer's disease participants (n = 374) from the Alzheimer's Disease Neuroimaging Initiative. We also compared Aβ measurements in a separate group with serial CSF measurements over 3.1 ± 0.8 years that preceded a single florbetapir session. Additional biomarker and cognitive data allowed us to further examine profiles of discordant cases.
Results: Florbetapir and CSF Aβ were inversely correlated across all diagnostic groups, and dichotomous measurements were in agreement in 86% of subjects. Among subjects showing the most disagreement, the 2 discordant groups had different profiles: the florbetapir$^+$/CSF Aβ^- group was larger (n = 13) and was made up of only normal and early MCI subjects, whereas the florbetapir$^-$/CSF Aβ^+ group was smaller (n = 7) and had poorer cognitive function and higher CSF tau, but no ApoE4 carriers. In the longitudinal sample, we observed both stable longitudinal CSF Aβ trajectories and those actively transitioning from normal to abnormal, but the final CSF Aβ measurements were in good agreement with florbetapir cortical retention.
Interpretation: CSF and amyloid PET measurements of Aβ were consistent in the majority of subjects in the cross-sectional and longitudinal populations. Based on our analysis of discordant subjects, the available evidence did not show that CSF Aβ regularly becomes abnormal prior to fibrillar Aβ accumulation early in the course of disease.

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The β-amyloid (Aβ) peptide is the primary component of neuritic plaques in Alzheimer disease (AD) and can be quantified in humans using cerebrospinal fluid (CSF) and positron emission tomography (PET) imaging measurements. A number of recent studies have reported that greater fibrillar Aβ in cortex, which has been measured previously with amyloid PET imaging using the tracer ^{11}C-Pittsburgh compound B (PiB), is associated with low concentrations of CSF $A\beta_{1-42}$ in normal aging and dementia.[1–7] Although this inverse relationship is consistent at the group level, there is not perfect agreement between the 2 markers, because some individuals with abnormal CSF $A\beta_{1-42}$ have normal amyloid PET and vice versa.[3] Specifically, some studies have suggested that when there is a discrepancy, CSF $A\beta_{1-42}$ may be more likely than amyloid PET to be abnormal in cognitively normal older individuals, leading to the possibility that CSF Aβ abnormalities precede fibrillar Aβ

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aggregation in cortex.[2,8,9] However, conflicting findings have also been reported,[6,10] indicating that further research is needed to understand how often and under what circumstances discordance between the 2 Aβ markers occurs.

The goal of this study was to examine the agreement between Aβ markers in normal aging, mild cognitive impairment (MCI), and AD. The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a large multisite study that includes a number of biomarkers, including CSF and amyloid PET imaging with the ^{18}F-labeled radioligand florbetapir. We evaluated 2 samples of ADNI participants: a large sample (n = 374) with concurrent florbetapir and CSF measurements, and a separate smaller sample (n = 60) with serial CSF measurements over approximately a 3-year period and ending prior to a single florbetapir scanning session. Based on previous studies, we expected to find evidence that abnormal Aβ can be detected in CSF prior to amyloid PET imaging, particularly in individuals with minimal or no cognitive deficits. We further predicted that other CSF, neuroimaging, genetic, and cognitive data in discordant cases would provide additional support for potentially differing roles of Aβ markers at different stages of disease severity.

Subjects and Methods

ADNI

Our study samples were drawn from different phases of the ADNI, a longitudinal multisite study supported by the National Institutes of Health, private pharmaceutical companies, and nonprofit organizations with approximately 50 medical center and university sites across the United States and Canada (http://adni.loni.ucla.edu). Subjects in this report are ADNI participants with either cross-sectional CSF and florbetapir measurements, or longitudinal CSF measures with a single florbetapir time point.

Full inclusion/exclusion criteria are described in detail at www.adni-info.org. Briefly, all subjects were between the ages of 55 and 90 years, had completed at least 6 years of education, were fluent in Spanish or English, and were free of any other significant neurologic diseases. Participants with MCI, now referred to as late MCI (LMCI), had a subjective memory complaint, a Clinical Dementia Rating (CDR) of 0.5, and were classified as single- or multidomain amnestic.[11] An early MCI (EMCI) group differed from LMCI only based on education-adjusted scores for the delayed paragraph recall subscore on the Wechsler Memory Scale-Revised Logical Memory II such that EMCI subjects were intermediate to normal subjects and LMCI. Normal subjects had CDR scores of 0, and patients with AD met standard diagnostic criteria.[12]

Participants

Our cross-sectional sample was made up of 374 subjects (103 normal, 187 EMCI, 62 LMCI, 22 AD at the time of the florbetapir scan; Table 1) who each had a single lumbar puncture (LP) and a florbetapir session between May 2010 and March 2012. LPs and florbetapir scans occurred within 2 weeks of each other (see Table 1).

Our longitudinal sample was made up of the 60 ADNI subjects (29 normal, 31 MCI at enrollment) who underwent an average of 3.5 LPs (minimum = 2, maximum = 5) at approximately yearly intervals between October 2005 and November 2010, and subsequently underwent florbetapir scanning an average of 1.4 $\pm$ 0.6 years after the last LP. The majority of subjects had concurrent structural magnetic resonance images (MRI) and fluorodeoxyglucose (FDG) scans, CSF tau and phosphorylated tau measurements, and cognitive function (eg, Mini-Mental State Examination [MMSE], Alzheimer's Disease Assessment Scale–Cognitive Subscale [ADAS-cog]).

Over the approximately 5-year follow-up period, 5 of 29 (17%) normal subjects converted to MCI, whereas 16 of 31 (52%) MCI subjects converted to AD and 3 of 31 (10%) MCI subjects reverted to normal status (Table 2).

All participants gave written informed consent that was approved by the internal review board of each participating institution.

Florbetapir Imaging and Analysis

Florbetapir image data were acquired from a variety of PET scanners and sites nationwide. Briefly, image data were acquired in four 5-minute frames 50 to 70 minutes after injection of approximately 10 mCi, and the 4 frames were coregistered to each other, averaged, interpolated to a uniform image and voxel size (160 $\times$ 106 $\times$ 96, 1.5 mm^3), and smoothed to a uniform resolution (8mm full width at half-maximum) to account for differences between scanners.[13]

To quantify cortical Aβ, preprocessed florbetapir image data and coregistered structural MRI were analyzed using Freesurfer v4.5.0 (http://surfer.nmr.mgh.harvard.edu/) as described elsewhere[14,15]. We used 1 or, in most cases, 2 T1 structural 1.5T or 3T MRI scans that were acquired as close as possible to the florbetapir scan to define cortical regions of interest, which were averaged together, coregistered to the florbetapir images to extract mean cortical retention, and then normalized to a cerebellar reference region as a summary measure of florbetapir retention for each subject.

CSF Data Analysis

LPs were carried out at designated ADNI sites. The CSF Aβ_{1-42}, total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau$_{181p}$) were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use–only reagents) immunoassay kit–based reagent as described and validated previously.[16–18] Additional analysis details and quality control procedures appear online (http://adni.loni.ucla.edu).

All longitudinal and cross-sectional CSF aliquots were anchored to the same baseline assay to use the cutoff values for abnormal and normal Aβ_{1-42}, t-tau, and p-tau$_{181p}$ status that

TABLE 1. Demographic and Descriptive Biomarker Information for the Cross-Sectional Study Population

Characteristic/Biomarker	Total Cross-Sectional Sample	Diagnosis at Florbetapir			
		Normal	EMCI	LMCI	AD
Subject characteristics					
No.	374	103	187	62	22
Time from LP to florbetapir, mo	0.4 (0.6)	0.3 (0.5)	0.4 (0.7)	0.3 (0.4)	0.3 (0.4)
Age at florbetapir, yr	72.7 (7.4)	74.9 (5.5)	71.3 (7.6)	72.5 (7.2)	74.6 (10.7)
Female sex, %	45	47	44	48	36
Education, yr	16.2 (2.7)	16.6 (2.6)	15.8 (2.6)	16.5 (2.6)	15.9 (2.7)
MMSE at florbetapir, range: 0–30	27.9 (2.3)	29 (1.1)	28.2 (1.6)	27.4 (2.1)	21.8 (2.8)
ADAS-cog at florbetapir, range: 0–70	8.9 (5.1)	6.3 (3)	8 (3.7)	12.1 (4.8)	19.6 (6.3)
Abnormal biomarkers, %					
ApoE4 carriers	38	21	39	55	64
Abnormal florbetapir	46	34	41	68	77
Abnormal CSF $A\beta_{1–42}$	43	31	36	68	86
Abnormal CSF tau	31	23	25	50	68
Abnormal CSF p-tau_{181p}	44	36	38	68	68
Abnormal FDG	32	17	26	49	100

$A\beta$ = β-amyloid; AD = Alzheimer disease; ADAS-cog = Alzheimer's Disease Assessment Scale–Cognitive Subscale; CSF = cerebrospinal fluid; EMCI = early mild cognitive impairment; FDG = fluorodeoxyglucose; LMCI = late mild cognitive impairment; LP = lumbar puncture; MMSE = Mini-Mental State Examination, Standard deviations are in parentheses.

were established and validated for that assay[17]; details are provided in the Supplementary Materials.

Additional Biomarkers and Cognitive Tests

Information about measurement of additional biomarkers (ApoE4, hippocampal volume, FDG PET) and neuropsychological testing appears in the Supplementary Materials.

Biomarker Cutoffs

Subjects were categorized as abnormal (+) or normal (−) on florbetapir using a cortical retention ratio cutoff value of 1.11.[15] This value is based on the upper limit of the 95% confidence interval for the distribution of florbetapir values for young healthy controls[19] and is consistent with a separate autopsy-validated sample.[20] The CSF cutoffs from the autopsy-validated baseline assay used in this study were $A\beta_{1–42}$ = 192 pg/ml, t-tau = 93 pg/ml, and p-tau_{181p} = 23 pg/ml[17]; low $A\beta_{1–42}$ and high tau values were considered abnormal (+). Finally, to categorize subjects as abnormal (+) and normal (−) on FDG, we used a cutoff of 1.21 that was derived from a receiver operating characteristic analysis of normal and AD subjects in a separate ADNI population.[21]

Statistical Methods

All statistical tests were performed with SPSS v19.0 (IBM, Armonk, NY) and carried out at $\alpha = 0.05$.

Associations that included continuous CSF and florbetapir measurements were assessed using Spearman rank correlation coefficients (ρ) to account for the non-normally distributed nature of these amyloid measurements. Associations between ApoE4 carrier status and other dichotomous measurements were assessed with chi-square tests. The κ statistic was used to quantify agreement between dichotomous (+/−) measurements (CSF, florbetapir, FDG) relative to what would be expected by chance.

Results

Descriptive Information and Biomarker Associations in the Cross-Sectional Population

Demographic information for the 374 normal, EMCI, LMCI, and AD participants in the cross-sectional sample is summarized in Table 1. Age, education, and sex were similar across diagnostic groups, whereas MMSE and ADAS-cog performance declined across groups as diagnostic severity increased. The percent of ApoE4 allele carriers and the percentage of subjects categorized as abnormal (+) on each biomarker (florbetapir, CSF $A\beta_{1–42}$, t-tau, p-tau_{181p}, and FDG) also increased with diagnostic severity. Of these markers, FDG status was most consistent with diagnosis, with 17% of normal subjects and 100% of AD patients categorized as abnormal.

TABLE 2. Demographic and Descriptive Biomarker Information for the Longitudinal Study Population

Characteristic/Biomarker	Total Longitudinal Sample	Diagnosis at Enrollment	
		Normal	MCI
Subject characteristics			
No.	60	29	31
Age at florbetapir, yr	80.1 (6.0)	82.1 (4.4)	78.3 (6.8)
Time between first and last LP, yr	3.1 (0.8)	3.2 (0.8)	3.1 (0.8)
Number of LP samples	3.5 (0.7)	3.5 (0.6)	3.5 (0.8)
Time between last LP and florbetapir, yr	1.4 (0.6)	1.4 (0.6)	1.3 (0.6)
Sex, % female	42%	48%	35%
Education, yr	16.0 (3.0)	16.1 (3.3)	15.9 (2.7)
ApoE4 carriers, %	38	52	24
MMSE at florbetapir, range: 0–30	26.4 (4.2)	28.7 (1.6)	24.3 (4.7)
ADAS-cog at florbetapir, range: 0–70	12.1 (9.2)	6.3 (3.8)	17.5 (9.5)
Abnormal biomarkers, %			
ApoE4 carriers	38	24	52
Abnormal florbetapir	55	41	68
Abnormal CSF $A\beta_{1–42}$	62	55	68
Abnormal CSF tau	32	24	39
Abnormal CSF p-tau_{181p}	68	72	65
Abnormal FDG	51	31	70
Diagnosis at florbetapir scan, No.			
Normal	27	24	3
MCI	17	5	12
AD	16	0	16

Aβ = β-amyloid; AD = Alzheimer disease; ADAS-cog = Alzheimer's Disease Assessment Scale–Cognitive Subscale; CSF = cerebrospinal fluid; FDG = fluorodeoxyglucose; LP = lumbar puncture; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination.

Across all individuals, age was associated with continuous forms of biomarkers (florbetapir, CSF Aβ, t-tau, and p-tau, hippocampal volume, and FDG), whereas education was weakly correlated with FDG ($p = 0.04$) but not CSF t-tau, p-tau, $A\beta_{1–42}$, hippocampal volume, or age.

Cross-Sectional Associations between CSF Aβ and Florbetapir

The inverse relationship between continuous forms of concurrent CSF Aβ and florbetapir measurements for all diagnostic groups is plotted in Figure 1A, as well as cutoffs for abnormal and normal status ($+/-$) for each biomarker.

Using continuous measures, florbetapir was more closely correlated with CSF Aβ ($\rho = -0.74$) than with t-tau ($\rho = 0.51$) or p-tau ($\rho = 0.55$) across the entire sample. Similarly, within individual diagnostic groups, florbetapir associations were stronger with CSF Aβ (normal, $\rho = -0.67$; EMCI, $\rho = -0.72$; LCMI, $\rho = -0.61$; AD, $\rho = -0.41$) than with t-tau (normal, $\rho = 0.23$; EMCI, $\rho = 0.55$; LCMI $\rho = 0.57$; AD, $\rho = 0.17$) or p-tau (normal, $\rho = 0.28$; EMCI, $\rho = 0.60$; LCMI $\rho = 0.55$; AD, $\rho = 0.30$).

We also evaluated dichotomous forms of these biomarkers. The majority (62%) of normal subjects were negative for both florbetapir and CSF Aβ, and the majority of AD patients (77%) were positive for both (see Fig 1C). The proportion of subjects with agreement was stable across diagnostic groups (83–91%; $\kappa = 0.72$ overall; Table 3).

Agreement between florbetapir status ($+/-$) and status on other biomarkers (CSF t-tau and p-tau, FDG) was moderate (CSF t-tau, $\kappa = 0.42$; CSF p-tau,

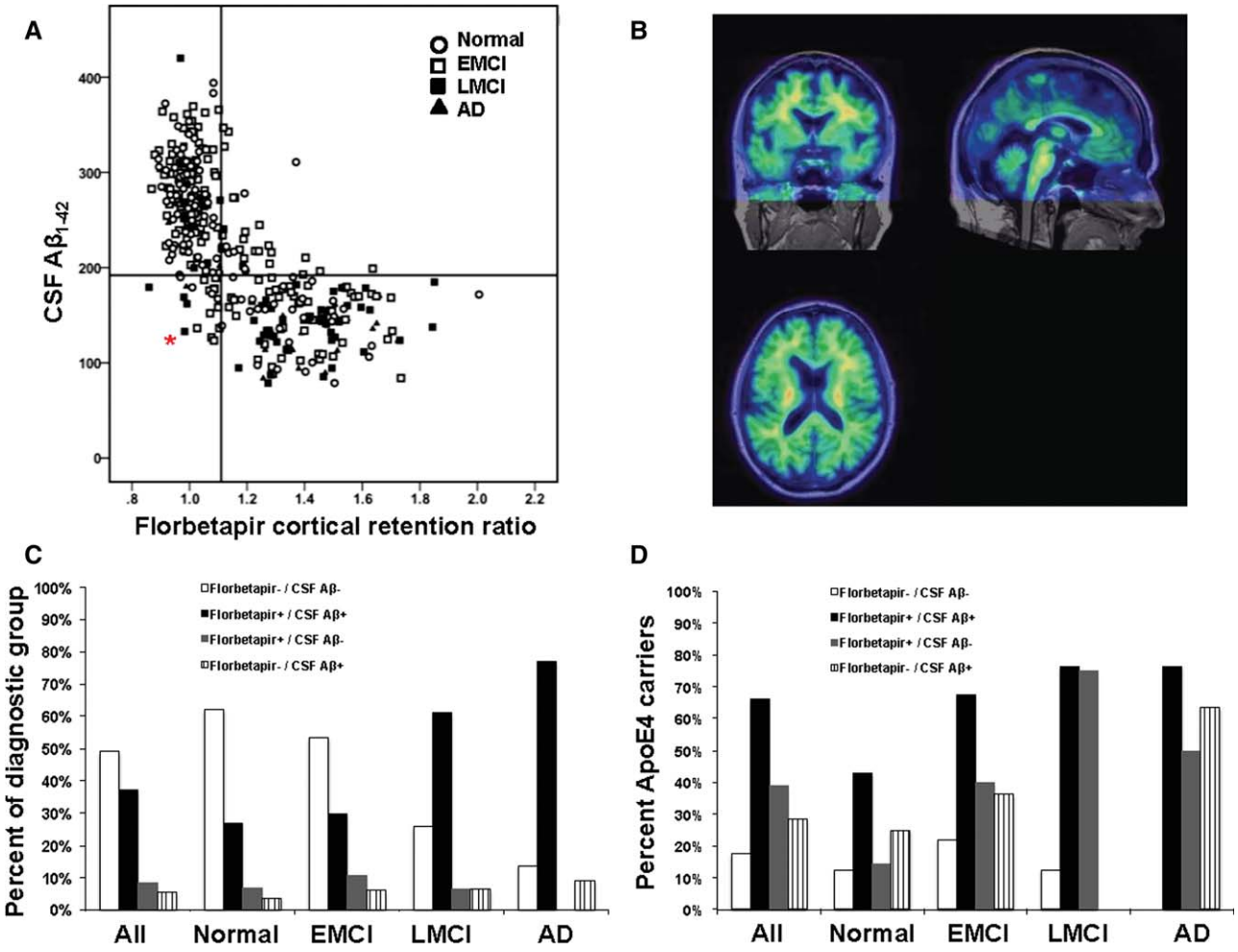

FIGURE 1: (A) The inverse association between florbetapir cortical retention ratios and cerebrospinal fluid (CSF) β-amyloid ($A\beta$)$_{1-42}$ is shown for normal, early mild cognitive impairment (EMCI), late mild cognitive impairment (LMCI), and Alzheimer disease (AD) individuals. Predefined cutoffs are shown for each marker (CSF $A\beta_{1-42}$ = 192pg/ml; florbetapir cortical retention ratio = 1.11) that were derived from independent samples (see Subjects and Methods). Subjects with concordant florbetapir and CSF $A\beta_{1-42}$ are in the upper left (florbetapir$^-$/CSF $A\beta^-$) and bottom right (florbetapir$^+$/CSF $A\beta^+$) quadrants, whereas subjects with discordant florbetapir and CSF $A\beta_{1-42}$ are in the upper right (florbetapir$^+$/CSF $A\beta^-$) and bottom left (florbetapir$^-$/CSF $A\beta^+$) quadrants. (B) A florbetapir scan for an example discordant LMCI subject (florbetapir$^-$/CSF $A\beta^+$) is shown (see *asterisk* on scatterplot in A), indicating that the visual read is consistent with the qualitative florbetapir measurement (florbetapir cortical retention ratio = 0.98) despite abnormal CSF status. (C) The percentage of individuals from each diagnostic group in each of the 4 scatterplot quadrants is shown in the bar graph. The proportion of subjects who are abnormal by both markers *(black bars)* increases as diagnostic severity increases, but the proportion of discordant subjects (*gray* and *striped bars*) is similar across diagnostic groups and between the 2 types of discordance. (D) The proportion of ApoE4 allele carriers who are concordant on both markers increases with diagnostic severity *(black bars)*; the proportion of ApoE4 carriers is moderate for the 2 discordant groups (*gray* and *striped bars*) in the normal and EMCI subjects.

κ = 0.52; FDG, κ = 0.26 for the total sample), but this was variable across diagnostic groups (see Table 3; also see Supplementary Fig).

The proportion of ApoE4 carriers was highest for subjects who were positive for both markers, lowest for subjects negative for both, and intermediate for the 2 discordant groups (see Fig 1D).

CSF Aβ and Florbetapir Disagreement

Across all diagnostic groups, 9 to 17% of subjects (52 subjects total; 31 florbetapir$^+$/CSF $A\beta^-$, 21 florbetapir$^-$/CSF $A\beta^+$) were discordant (see Fig 1C).

Visual inspection of florbetapir$^-$/CSF $A\beta^+$ indicated that the quantitative florbetapir estimates plotted in Figure 1A are consistent with qualitative interpretation (see Fig 1B).

To identify subjects who were considerably discordant, as opposed to those with 1 or both $A\beta$ measurements close to the cutoffs, we created $\pm$5% confidence intervals around each cutoff (Fig 2). Of the original 52 discordant subjects, 20 discordant subjects remained (13 florbetapir$^+$/CSF $A\beta^-$ and 7 florbetapir$^-$/CSF $A\beta^+$). The diagnoses, cognitive measurements, and imaging and fluid biomarker profiles of these remaining discordant

TABLE 3. κ Statistics Representing Agreement in +/− Status between Florbetapir and the Other Biomarkers in the Cross-Sectional Sample

Biomarker	Total Cross-Sectional Sample	Diagnosis at Florbetapir			
		Normal	EMCI	LMCI	AD
CSF $A\beta_{1-42}$	0.72	0.76	0.65	0.71	0.70
CSF tau	0.42	0.27	0.46	0.39	0.09
CSF p-tau_{181p}	0.52	0.45	0.52	0.48	0.32
FDG	0.26	0.21	0.11	0.33	[a]

[a] κ was invalid because all AD subjects were FDG^+.
$A\beta$ = β-amyloid; AD = Alzheimer disease; CSF = cerebrospinal fluid; EMCI = early mild cognitive impairment; FDG = fluorodeoxyglucose; LMCI = late mild cognitive impairment.

subjects are listed in Table 4. One hundred percent (13 of 13) of subjects in the $florbetapir^+$/CSF $A\beta^-$ group were in the 2 most cognitively intact groups (cognitively normal or early MCI). The $florbetapir^-$/CSF $A\beta^+$ group, conversely, had more cognitive impairment (5 of 7 subjects had a diagnosis of LMCI and AD) and higher CSF tau ($p = 0.01$) than the other discordant group, but a lower we created ±5% confidence proportion of ApoE4 carriers (0 of 7 subjects, compared with 6 of 13 [46%] in the $florbetapir^+$/CSF $A\beta^-$ group; chi-square test; $p = 0.03$). Group differences between the other biomarkers were not significant ($p > 0.10$).

Longitudinal CSF Aβ Trajectories for Florbetapir$^{+/-}$ Individuals

Demographic information for the longitudinally followed subjects is shown in Table 2. A number of subjects had changes in diagnosis during follow-up; 52% of MCI subjects converted to AD, and 17% of normal individuals progressed to MCI prior to the florbetapir scan.

CSF Aβ trajectories for the longitudinally followed, separate sample are plotted in Figure 3A. Subjects were divided by florbetapir status and by diagnosis at the time of florbetapir (end of CSF follow-up), so all AD subjects in Figure 3A were diagnosed as MCI at enrollment, and several normal and MCI subjects had a different diagnosis at enrollment as well (see Table 2). Unlike the cross-sectional population, the CSF Aβ measures occurred >1 year before florbetapir scans. Nonetheless, κ values reflecting agreement between the last CSF $A\beta^{+/-}$ status and $florbetapir^{+/-}$ status were similar to the cross-sectional data set (normal, $\kappa = 0.67$; MCI, $\kappa = 0.65$; AD, $\kappa = 0.82$). There were fluctuations in CSF Aβ over the

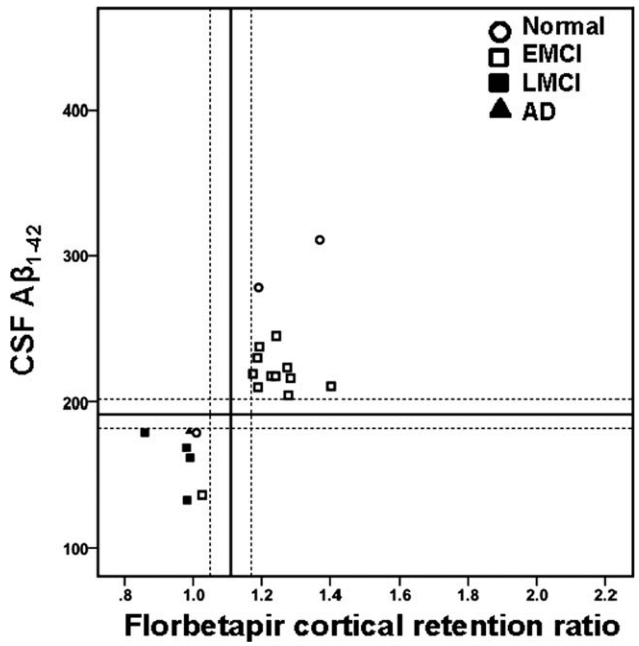

FIGURE 2: Subjects who remain discordant after applying a ±5% confidence interval to each cutoff. Aβ = β-amyloid; AD = Alzheimer disease; CSF = cerebrospinal fluid; EMCI = early mild cognitive impairment; LMCI = late mild cognitive impairment.

TABLE 4. Demographic, Cognitive, and Biomarker Profiles of Discordant Subjects Who Survived the 5% Cutoff Confidence Interval

Status	Dx	Age, yr	Sex	Education, yr	ApoE4	ADAS-cog	MMSE	AVLT	HV/ICV, $\times 10^{-3}$	Florbetapir	CSF $A\beta_{1-42}$	CSF tau	CSF ptau	FDG
Florbetapir^{+}/ CSF^{-}, n = 13	N	71	F	20	0	5	28	52	4.60	1.37 +	311	80	17	
		78	M	14	0	6	30	44	4.88	1.19 +	278	81	37	1.39
	EMCI	81	M	20	0	8	27	41	4.60	1.28 +	204	58	20	
		63	F	20	0	6	29	50	5.10	1.17 +	219	79	22	1.51
		73	F	12	1	5	30	46	5.87	1.40 +	211	139 +	53	1.19 (+)
		66	M	18	1	5	30	50	5.35	1.28 +	216	77	21	1.20 (+)
		62	F	20	1	8	29	55	4.40	1.19 +	230	50	22	1.39
		71	M	20	0	5	29	36	5.36	1.24 +	217	90	19	1.35
		68	F	14	1	7	27	41	4.50	1.19 +	238	85	22	1.45
		75	M	20	1	9	29	31	5.66	1.19 +	210		23 (+)	1.18 (+)
		82	F	14	0	7	29	26	5.06	1.28 +	223	111 +	26 +	1.24
		74	F	13	0	6	28	37	5.64	1.24 +	245	42	19	1.35
		64	M	18	1	9	27	34	3.36	1.23 +	218	81	24 (+)	1.11 +
Florbetapir^{-}/ CSF^{+}, n = 7	N	86	F	12	0	6	26	35	4.94	1.01	179 +	120 +	26 +	1.18 (+)
	EMCI	70	M	18	0	6	28	30	4.03	1.03	137 +	38	25 +	1.40
	LMCI	72	M	19	0	8	30	42	5.14	0.98	133 +	60	20	1.47
		67	M	17	0	11	30	35	5.01	0.99	162 +	140 +	31 +	1.44
		80	F	16	0	10	26	36	3.76	0.98	169 +	47	24 (+)	1.11 +
		91	M	20	0	13	27	36	3.77	0.86	179 +	38	11	1.18 (+)
	AD	76	M	16	0	26	18	19	4.25	0.99	180 +	146 +	27 +	1.13 +

Subjects whose positron emission tomography and CSF measurements were abnormal based on previously derived cutoffs are indicated: + = abnormal, (+) = borderline (see Subjects and Methods).

Aβ = β-amyloid; AD = Alzheimer disease; ADAS-cog = Alzheimer's Disease Assessment Scale–Cognitive Subscale; AVLT = Auditory Verbal Learning Test; CSF = cerebrospinal fluid; Dx = diagnosis; EMCI = early mild cognitive impairment; F = female; FDG = fluorodeoxyglucose; HV = hippocampal volume; ICV = intracranial volume; LMCI = late mild cognitive impairment; M = male; N = normal.

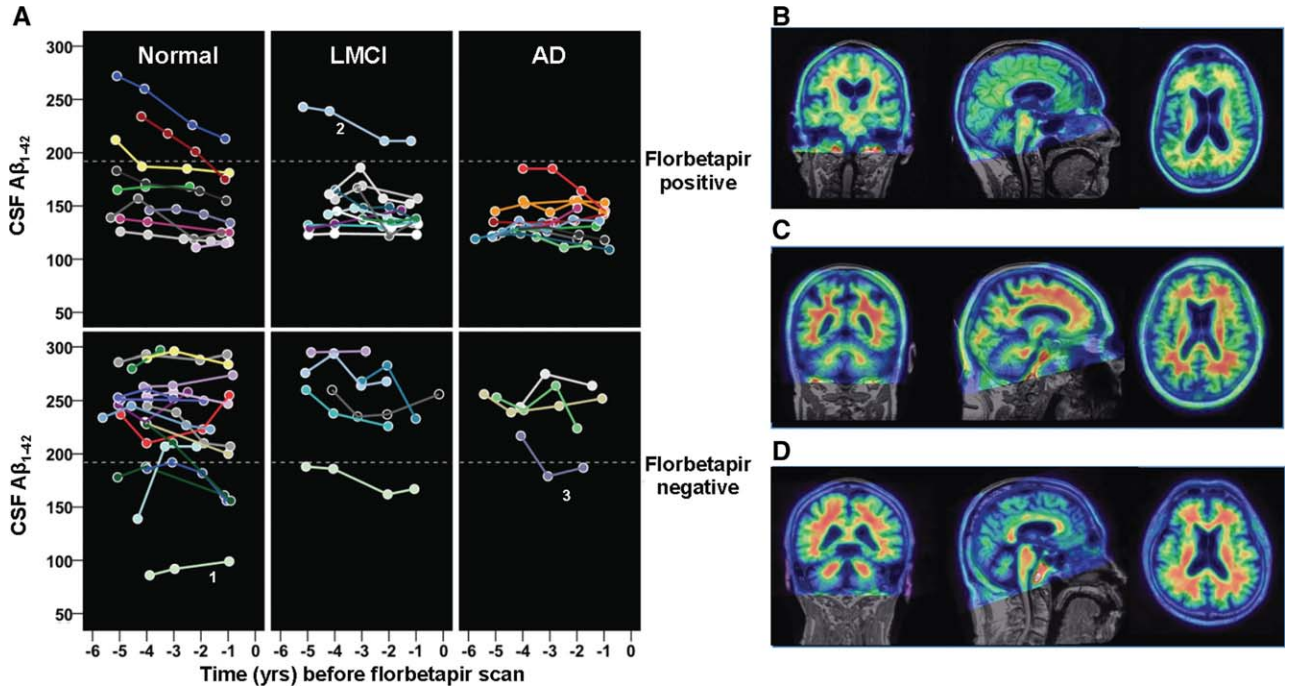

FIGURE 3: (A) Longitudinal cerebrospinal fluid (CSF) *β*-amyloid ($A\beta_{1-42}$) data are plotted against time for each subject in the longitudinal sample, with florbetapir$^+$ individuals in the top row and florbetapir$^-$ subjects in the bottom row. Subjects are plotted separately by diagnosis at the time of florbetapir (*left column,* 27 normal; *middle column,* 17 late mild cognitive impairment [LMCI]; *right column,* 16 Alzheimer disease [AD]). Time of zero corresponds to the florbetapir scan, each colored line corresponds to an individual subject, and each point on the line corresponds to a CSF $A\beta_{1-42}$ value from a single lumbar puncture. Dotted lines in each panel represent the CSF $A\beta_{1-42}$ cutoff value (192pg/ml) that divides abnormal values (below line) from normal values (above line). In the top panel, CSF A*β* values that are concordant with florbetapir appear below the dotted line (both abnormal), whereas in the bottom panel CSF A*β* values that are concordant with florbetapir appear above the dotted line (both normal). (B–D) Representative florbetapir scans are shown for 3 discordant subjects: (B) a CSF $A\beta^+$/florbetapir$^-$ normal 80-year-old male (florbetapir cortical retention ratio = 1.06, labeled 1 in A), (C) a CSF $A\beta^-$/florbetapir$^+$ 84-year-old MCI male (florbetapir cortical retention ratio = 1.12, labeled 2 in A), and (D) a CSF $A\beta^+$/florbetapir$^-$ 81-year-old AD male (florbetapir cortical retention ratio = 0.99, labeled 3 in A). All longitudinal CSF samples for an individual subject were included in the same immunoassay analytical run to minimize variance due to run-to-run and reagent lot-to-lot variabilities.

course of follow-up for many subjects, but florbetapir$^+$ individuals (see Fig 3A, *top row*) had primarily downward CSF A*β* trajectories. Four subjects had normal CSF A*β* at enrollment and declines throughout the 6-year follow-up, ending with abnormal—or near abnormal—measurements that preceded abnormal florbetapir status. Although the gap between the last CSF measurement and the florbetapir scan leaves some uncertainty, the direction of change for these actively transitioning subjects suggests good agreement between coinciding CSF A*β* and florbetapir.

There were, however, several discordant subjects whose florbetapir scans appear in Figure 3B–D (see the Supplementary Materials for additional demographic and biomarker characteristics).

Discussion

We found that CSF $A\beta_{1-42}$ and amyloid PET imaging measurements were inversely associated in the majority of subjects, and that dichotomous classification was in substantial agreement. There was no evidence from cross-sectional or longitudinal analyses that abnormal CSF A*β* precedes abnormal florbetapir early in the course of disease.

We observed good agreement between CSF A*β* and amyloid PET measurements across several comparisons: with continuous or dichotomous forms of the variables, using cross-sectional and longitudinal CSF measurements, and across diagnostic groups. Overall, the association between CSF A*β* and florbetapir explains approximately 55% of the variance in these measurements, which is comparable to previous studies with PiB.[2–7] As expected, the proportion of subjects who were abnormal on both markers increased with severity of diagnosis, but the overall proportion of subjects who had concordant (both normal or both abnormal) and discordant A*β* measurements was stable across diagnostic groups (83–91% concordant, 9–17% discordant). In the longitudinal CSF sample, there was considerable change in CSF A*β* from the beginning to the end of the follow-up period for some subjects, with most change occurring in

a decreasing direction. Consistent with the cross-sectional sample, there was good agreement between the final CSF Aβ and the subsequent florbetapir measurement.

Our data did not support the hypothesis that a decline in CSF Aβ precedes aggregation of fibrillar Aβ.[2,8] Among discordant subjects whose measurements were not close to the cutoffs, normal and EMCI subjects made up 100% of the CSF Aβ^-/florbetapir$^+$ group but only 29% of the CSF Aβ^+/florbetapir$^-$ group (see Fig 2). Because active accumulation of amyloid is most likely to occur prior to the onset of significant cognitive decline,[22,23] our findings support the possibility that fibrillar Aβ can be detected first in some individuals, which has been reported,[6,10] or that there is a complex relationship between different species of Aβ and the progression of disease. For example, although decreasing CSF Aβ measurements in AD are generally thought to reflect the accumulation of soluble forms of Aβ in neuritic plaques,[24,25] this process may be altered by comorbid pathology or other etiologies that influence the production or clearance of different Aβ species. Specifically, low CSF Aβ in the absence of neuritic plaques has been reported in other disorders such as amyotrophic lateral sclerosis and Creuzfeldt–Jacob syndrome.[26] Detection may play an important role as well; a recent case study reported low CSF Aβ in the presence of diffuse plaques detected at autopsy but not with PiB PET imaging.[27] Although more longitudinal studies are needed, the existing evidence suggests that there may be considerable variability in the temporal dynamics and pattern of soluble and fibrillar Aβ.

Nonetheless, the combination of florbetapir and CSF marker information provided useful insight into diagnostic status for some subjects. For example, 3 of 22 subjects in the cross-sectional sample were diagnosed with AD at enrollment but were negative for both markers, indicating that their dementia is likely due to non-AD pathology. Similarly, in the longitudinal sample, 4 of 31 MCI subjects converted to AD during the follow-up period but were negative for both markers (see Fig 3A; 1 was borderline positive for CSF Aβ). Misdiagnosis in AD patients with normal CSF Aβ and amyloid PET has been suggested previously[28] and may account for some amyloid-negative AD subjects in this study. Furthermore, comorbidities may have influenced the accuracy of the biomarker cutoffs themselves, and may account for inaccuracies in both clinical diagnoses and biomarker classifications. Of subjects who have come to autopsy, 5 of 9 ADNI MCI and AD subjects (not in this study) had comorbid pathologies such as α-synuclein pathology and tauopathy.[29] Furthermore, in a recent study of dementia patients that included individuals with comorbidities, the sensitivity and specificity of CSF biomarker measurements was lower for clinical compared with neuropathological diagnosis,[30] providing additional evidence that both misdiagnosis and non-AD pathology influence biomarker accuracy.

The longitudinal sample provided additional insight into the relationship between the 2 markers and the time course of the accumulation of amyloid pathology. Whereas minimal longitudinal change in serial CSF Aβ measurements in normal or AD individuals has been reported previously,[1,31] we observed a combination of stable trajectories and considerable variability and declines for some individuals. CSF Aβ trajectories were variable over time for those who were florbetapir$^-$ at the end of follow-up, but there was minimal net change. Among those who were ultimately florbetapir$^+$, we observed several individuals whose CSF Aβ actively declined throughout the 5- to 6-year follow-up period to levels that were abnormal or close to abnormal, and this status was ultimately reflected by their abnormal florbetapir scan as well. We note that there is ambiguity about whether CSF Aβ became abnormal before florbetapir or vice versa due to the approximately 1-year delay between the final CSF measurement and the florbetapir scan; however, the downward trajectory of CSF measurement appears to be informative.

Older age in our sample may account for why we did not find evidence that CSF Aβ becomes abnormal prior to amyloid PET measurements. Previous cross-sectional PiB studies suggesting a possible offset in the time course of Aβ abnormality had subjects as young as 43 years (and a mean age in the mid 60s).[2,8] Studies that did not report a pattern that was consistent with CSF Aβ becoming abnormal prior to amyloid PET had mean ages of approximately 65 years[10] and 71 years,[6] whereas the subjects in the current study had a mean age of 73 years. Because Aβ aggregation may begin earlier than 50 years of age,[32] our subjects may have passed a critical time period where the offset would be most clearly observed. Older age in our population may also explain why we did not find any evidence for an initial increase in CSF Aβ followed by a subsequent decline, although to our knowledge this has only been reported in autosomal dominant AD,[22,33] and not in late onset AD.[34]

Several other methodological factors may have contributed to our findings. Although we had a large sample overall, the relatively small numbers of discordant subjects (particularly in the longitudinal sample) made it difficult to draw conclusions about the cause of the discordance. Disagreement between CSF Aβ and florbetapir measurements may have been due to measurement problems such as errors introduced by PET image processing, the use of cutoffs with differing sensitivities and specificities, and standardizing CSF assays to the same set

of cutoffs. Establishing standardization across laboratories for LP collection and CSF assay analysis is a significant challenge that is currently being addressed.[18,35] In addition, the cutoffs and distributions CSF Aβ and florbetapir differ in a way that influences the shape and linearity of their association. Both markers have an approximately bimodal distribution across the entire sample, but for florbetapir the broadest part of the distribution relative to the cutoff is in the abnormal range of values, whereas for CSF Aβ the broadest part of the distribution is in the normal range of values, resulting in a nonlinear inverse relationship when they are plotted against each other.

Overall, we found good agreement between florbetapir and CSF Aβ, and we did not find any evidence that CSF Aβ is more likely to become abnormal prior to the accumulation of fibrillar Aβ early in the course of disease. Furthermore, disagreement between Aβ measurements was not uncommon. One in 7 individuals in this study (or 1 in 20 after applying cutoff confidence intervals) had discordant Aβ markers and is therefore considered an ambiguous case according to recently revised AD diagnostic criteria.[36] Understanding discrepancies between in vivo Aβ measurement is important, because the new criteria treat these markers as interchangeable in terms of diagnostic utility. In addition, in vivo Aβ measurement to aid in development and testing of pharmaceutical treatments targeting Aβ is underway, making accurate measurement an essential component of subject enrichment and evaluation of drug efficacy in clinical trials. Future research may address remaining questions about the relationship between different species of Aβ. Forthcoming longitudinal data in the current sample will be critical for determining the clinical relevance of these imbalances.

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Potential Conflicts of Interest

S.M.L.: consultancy, Avid Radiopharmaceuticals, Synarc, Biogen Idec, Janssen Alzheimer Immunotherapy; employment, Avid Pharmaceuticals; travel expenses, ADNI. M.P.: employment, Eli Lilly. J.Q.T.: may accrue revenue on patents submitted by the University of Pennsylvania wherein he is coinventor and he received revenue from the sale of Avid to Eli Lily as coinventor on imaging-related patents submitted by the University of Pennsylvania. L.M.S.: speaking fees, travel expenses, Siemens; consultancy, Innogenetics, ADNI. W.J.J.: consultancy, Genentech, Elan/Janssen Alzheimer Immunotherapy, TauRx, Siemens, Synarc, Avid Radiopharmaceuticals, ADNI, GE Healthcare.

References

1. Buchhave P, Blennow K, Zetterberg H, et al. Longitudinal study of CSF biomarkers in patients with Alzheimer's disease. PLoS One 2009;4:e6294.
2. Fagan AM, Mintun MA, Shah AR, et al. Cerebrospinal fluid tau and ptau(181) increase with cortical amyloid deposition in cognitively normal individuals: implications for future clinical trials of Alzheimer's disease. EMBO Mol Med 2009;1:371–380.
3. Jagust WJ, Landau SM, Shaw LM, et al. Relationships between biomarkers in aging and dementia. Neurology 2009;73:1193–1199.
4. Weigand SD, Vemuri P, Wiste HJ, et al. Transforming cerebrospinal fluid Abeta42 measures into calculated Pittsburgh compound B units of brain Abeta amyloid. Alzheimers Dement 2011;7:133–141.
5. Grimmer T, Riemenschneider M, Forstl H, et al. Beta amyloid in Alzheimer's disease: increased deposition in brain is reflected in reduced concentration in cerebrospinal fluid. Biol Psychiatry 2009;65:927–934.

6. Koivunen J, Pirttila T, Kemppainen N, et al. PET amyloid ligand [11C]PIB uptake and cerebrospinal fluid beta-amyloid in mild cognitive impairment. Dement Geriatr Cogn Disord 2008;26:378–383.
7. Tolboom N, van der Flier WM, Yaqub M, et al. Relationship of cerebrospinal fluid markers to 11C-PiB and 18F-FDDNP binding. J Nucl Med 2009;50:1464–1470.
8. Morris JC, Roe CM, Xiong C, et al. APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. Ann Neurol 2010;67:122–131.
9. Fagan AM, Mintun MA, Mach RH, et al. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. Ann Neurol 2006;59:512–519.
10. Forsberg A, Almkvist O, Engler H, et al. High PIB retention in Alzheimer's disease is an early event with complex relationship with CSF biomarkers and functional parameters. Curr Alzheimer Res 2010;7:56–66.
11. Petersen RC. Conceptual overview. In: Petersen RC, ed. Mild cognitive impairment: aging to Alzheimer's disease. New York, NY: Oxford University Press, 2003:1–14.
12. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34: 939–944.
13. Joshi A, Koeppe RA, Fessler JA. Reducing between scanner differences in multi-center PET studies. Neuroimage 2009;46:154–159.
14. Mormino EC, Kluth JT, Madison CM, et al. Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects. Brain 2009;132(pt 5):1310–1323.
15. Landau SM, Mintun M, Joshi A, et al. Amyloid deposition, hypometabolism, and longitudinal cognitive decline. Ann Neurol 2012;72:578–586.
16. Olsson A, Vanderstichele H, Andreasen N, et al. Simultaneous measurement of beta-amyloid(1–42), total tau, and phosphorylated tau (Thr181) in cerebrospinal fluid by the xMAP technology. Clin Chem 2005;51:336–345.
17. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 2009;65:403–413.
18. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI. Acta Neuropathol 2011;121:597–609.
19. Joshi AD, Pontecorvo MJ, Clark CM, et al. Performance characteristics of amyloid PET with florbetapir F 18 in patients with Alzheimer's disease and cognitively normal subjects. J Nucl Med 2012;53:378–384.
20. Clark CM, Schneider JA, Bedell BJ, et al. Use of florbetapir-PET for imaging beta-amyloid pathology. JAMA 2011;305:275–283.
21. Landau SM, Harvey D, Madison CM, et al. Comparing predictors of conversion and decline in mild cognitive impairment. Neurology 2010;75:230–238.
22. Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012;367:795–804.
23. Jack CR Jr, Vemuri P, Wiste HJ, et al. Evidence for ordering of Alzheimer disease biomarkers. Arch Neurol 2011;68:1526–1535.
24. Motter R, Vigo-Pelfrey C, Kholodenko D, et al. Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. Ann Neurol 1995;38:643–648.
25. Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. Neurology 2003;60:652–656.
26. Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol 2003;2:605–613.
27. Cairns NJ, Ikonomovic MD, Benzinger T, et al. Absence of Pittsburgh compound B detection of cerebral amyloid beta in a patient with clinical, cognitive, and cerebrospinal fluid markers of Alzheimer disease: a case report. Arch Neurol 2009;66:1557–1562.
28. Shimada H, Ataka S, Takeuchi J, et al. Pittsburgh compound B-negative dementia: a possibility of misdiagnosis of patients with non-Alzheimer disease-type dementia as having AD. J Geriatr Psychiatry Neurol 2011;24:123–126.
29. Cairns NJ, Taylor-Reinwald L, Morris JC. Autopsy consent, brain collection, and standardized neuropathologic assessment of ADNI participants: the essential role of the neuropathology core. Alzheimers Dement 2010;6:274–279.
30. Toledo JB, Brettschneider J, Grossman M, et al. CSF biomarkers cutoffs: the importance of coincident neuropathological diseases. Acta Neuropathol 2012;124:23–35.
31. Bouwman FH, van der Flier WM, Schoonenboom NS, et al. Longitudinal changes of CSF biomarkers in memory clinic patients. Neurology 2007;69:1006–1011.
32. Kok E, Haikonen S, Luoto T, et al. Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. Ann Neurol 2009;65:650–657.
33. Reiman EM, Quiroz YT, Fleisher AS, et al. Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. Lancet Neurol 2012;11:1048–1056.
34. Fagan AM, Roe CM, Xiong C, et al. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol 2007;64:343–349.
35. Vanderstichele H, Bibl M, Engelborghs S, et al. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. Alzheimers Dement 2012;8:65–73.
36. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–269.