Peripheral Aβ subspecies as risk biomarkers of Alzheimer's disease

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Plasma Aβ42 and Aβ40 levels are putative biomarkers for Alzheimer's disease (AD), but their significance and predictive value have been inconclusive. In AD transgenic models, plasma and cerebrospinal fluid levels of Aβ42 and Aβ40 increase with age but subsequently decrease when Aβ begins to accumulate in brain and with the onset of cognitive impairment. To determine the predictive value of Aβ levels in elderly populations, we investigated how plasma Aβ42, Aβ40, and a protofibrillar subspecies of Aβ42 changed over time and with the onset of cognitive impairment or AD. In a cohort of 1,125 elderly persons without dementia, 104 (9.2%) of the participants developed AD over 4.6 years of follow-up. Higher plasma Aβ42 levels at the onset of the study were associated with a threefold increased risk of AD. However, conversion to AD was accompanied by a significant decline in plasma Aβ42, a decreased Aβ42/Aβ40 ratio and, with the onset of cognitive impairment, decreased protofibrillar Aβ42 levels. Our results suggest individuals with elevated plasma Aβ42 are at increased risk of AD and that with the onset of disease, the decline in some forms of Aβ may reflect compartmentalization of Aβ peptides in the brain.

plasma amyloid beta40 and beta42 | protofibrillar Abeta

Amyloid β-peptides Aβ40 and Aβ42 are the two major species generated by sequential proteolytic cleavage by β- and δ-secretases of the amyloid precursor protein (APP) (1). Subsequent deposition of Aβ42 has been considered an initial and critical step in the pathogenesis of Alzheimer's disease (AD). Brain levels of Aβ42 increase with the development of dementia and are correlated with cognitive decline (2). Mutations in the *APP* and presenilin (*PSEN*1 and 2) genes, which result in a dominantly inherited form of early-onset AD, are accompanied by an increase in plasma Aβ42 and Aβ40 levels in patients before disease onset and are elevated in their unaffected relatives as well (3–5). Furthermore, both Aβ42 and Aβ40 plasma levels are increased in cognitively normal first-degree relatives of cases with late-onset AD without known mutations (6).

The relation between brain and plasma Aβ in health and disease is complex, but studies of AD transgenic mice have provided some insights. Within a few months of birth, APP transgenic mice secreted more Aβ42 and Aβ40 than their wild-type littermates (7, 8), and Aβ secretion is increased throughout the life of APP transgenic mice. Both plasma and cerebrospinal fluid (CSF) levels of Aβ42, and to a lesser extent Aβ40, increase with age, but both precipitously decrease as Aβ42 and Aβ40 levels rise in the brain (7). By 1 year, there are frank Aβ plaques, and subsequently there are characteristic age-related behavioral changes in memory. However, Lesne and colleagues (9) reported that the behavioral effects are not the result of the Aβ accumulation in brain, but the increase in the protofibrillar subspecies of Aβ.

These observations in mice represent a model for changes in plasma Aβ as a biomarker associated with AD in humans. Among nondemented elderly, plasma Aβ42 has been found to be increased in women with mild cognitive impairment (10), in adults with Down syndrome who subsequently develop dementia (11, 12), and in healthy elderly before late-onset AD (13, 14). In nondemented elderly, plasma Aβ42 levels were elevated and then declined over time, with a corresponding decline in a cognitive-screening test score (15). CSF Aβ42 and Aβ40 are reduced in those with cognitive impairment or very mild AD and when the disease is established (16). In addition, levels of CSF Aβ42 are inversely related to brain amyloid load as imaged with Pittsburgh Compound B (17, 18). Based on these results, it has been proposed that plasma levels of Aβ42 increase before the onset of AD and decline shortly after the onset of dementia and with progression of symptoms (10, 13, 19–21), although decline in CSF Aβ42 precedes cognitive decline associated with AD (17, 18). However, these observations are not consistent across studies, and the relation of Aβ peptides to AD has been attributed to the effects of age (22), high levels of Aβ40 (23), or a low plasma Aβ42/Aβ40 ratio (24). It is possible that the variability in the reports concerning plasma Aβ42 and Aβ40 as a biomarker of risk for AD may be related to when the plasma measurements are obtained and whether there is a concomitant accumulation of the protofibrillar forms of Aβ.

We evaluated the use of plasma Aβ42 and Aβ40 as biomarkers for AD in a longitudinal study of elderly participants from northern Manhattan in New York City. Plasma samples were obtained ≈4.5 years apart. None of the participants were demented at the inception of the study. In addition to measuring plasma Aβ42 and Aβ40, in a subset we also measured protofibrillar Aβ42 by using a newly developed antibody and total soluble Aβ by using a commercially available antibody 4G8.

Results

Demographic Characteristics. The mean duration of follow-up was 4.6 (± 0.7) years, and over the course of follow-up, 104 participants (9.2%) developed AD. Baseline levels of plasma Aβ40 and Aβ42 were correlated with each other ($r = 0.68$, $P = 0.001$). Plasma Aβ40 and Aβ42 but not the Aβ42/Aβ40 ratio were modestly related to age among those who remained nondemented over the follow-up period (Aβ40: $r = 0.222$, $P = 0.001$; Aβ402: $r = 0.198$, $P = 0.001$; and Aβ42/Aβ40 ratio: $r = -0.065$, $P = 0.037$). However, the relation between Aβ peptides and age

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Table 1. Demographic characteristics

Demographic characteristic	No dementia	Incident AD
No.	1021	104
Age at baseline, years (mean ± SD)***	76.3 ± 6.1	80.7 ± 7.0
Sex, *n* (% women)	693 (67.9)	75 (72.1%)
Education, years (mean ± SD)***	10.4 ± 4.7	6.7 ± 4.8
Ethnicity, *n* (%)***	319 (97)	10 (3)
White	338 (91.6)	31 (8.4)
African American	364 (85.2)	63 (14.8)
Hispanic		
APOE ε4 allele, *n* (%)	265 (26.3%)	28 (27.2%)
AB40, baseline (mean ± SE)	79.6 ± 1.9	87.2 ± 5.4
AB40, follow up (mean ± SE)	120.3 ± 1.6	128.8 ± 4.5
AB42, baseline (mean ± SE)*	37.3 ± 0.77	42.2 ± 2.9
AB42 follow up (mean ± SE)	48.2 ± 0.7	47.9 ± 2.0
AB42/AB40 baseline (mean ± SE)	0.60 ± 0.01	0.64 ± 0.06
AB42/AB40 follow up (mean ± SE)	0.46 ± 0.01	0.46 ± 0.01

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

at baseline among those who subsequently developed AD was significant only for Aβ40 (Aβ40: $r = 0.235$, $P = 0.016$; Aβ42: $r = 0.103$, $P = 0.30$; and Aβ42/Aβ40 ratio: $r = 0.065$, $P = 0.51$). Compared with those who remained nondemented, those who developed AD were older, more likely to be African American or Hispanic than white Caucasians, and less well educated, but did not differ by sex or the presence of an APOE-ε4 allele (Table 1).

Relation of Initial Aβ Peptides to Incidence of AD. Mean Aβ42 but not Aβ40 levels were significantly higher at baseline in those who subsequently developed AD than in those who remained nondemented (Table 1). Participants in the two highest quartiles of plasma Aβ42 levels were two to three times more likely to develop AD than those in the lowest quartile [hazard ratio (HR) = 2.2, 95% C.I. of 1.1–4.7 for those in the second highest quartile and HR = 3.4, 95% C.I. of 1.6–7.6 for those in the highest quartile], whereas the risk of AD did not vary by quartile of Aβ40 level (Table 2). These associations did not change in the multivariate Cox regression model after adjustment for age at baseline, sex, ethnicity, education, body mass index (BMI), and the presence of the APOE ε4 allele. Quartiles of the ratio of Aβ42/Aβ40 at baseline were not related to risk of AD (Table 2).

Relation of Change in Aβ Peptide Levels to Incidence of AD. Decreases in Aβ42 levels but not Aβ40 levels were associated with a significant increase in the risk of conversion to AD over the follow-up period, both when changes in Aβ42 levels were assessed as a continuous variable and with respect to change groups (Table 3). Compared with those whose Aβ42 levels increased over the follow-up period, those with decreasing levels of Aβ42 were three times more likely to develop AD [Odds Ratio (OR) = 2.8, 95% C.I. of 1.6–5.1] (Fig. 1), whereas there was no association between decreasing levels of Aβ40 and the development of AD (OR = 0.6, 95% C.I. of 0.2–1.7) (Table 3). Decrease in the ratio of Aβ42/Aβ40 was also strongly related to the development of AD. Compared with those with an increasing Aβ42/Aβ40 ratio, those whose Aβ42/Aβ40 ratios did not change and those with a decreasing Aβ42/Aβ40 ratio were three times more likely to have progressed to AD during that time period (OR = 3.1, 95% C.I. of 1.0–10.1 for those in the no change group; OR = 3.6, 95% C.I. of 1.1–12.1 for those in the decreasing group) (Table 3). These associations did not change in multivariate logistic regression models, adjusting for age at baseline, sex, ethnicity, education, BMI, and the presence of the APOE ε4 allele (Table 3).

Protofibrillar Aβ and Mild AD. In a subset of 402 participants, we studied the relation of 13C3, an antibody to a protofibrillar form of Aβ42, to the development of mild AD and examined an antibody to total soluble Aβ, 4G8, a measure of overall Aβ burden. Protofibrillar Aβ42, as measured by 13C3 antibody, was detectable in 34% of the cohort; thus, 66% had no detectable protofibrillar Aβ in plasma. In contrast, >90% of the participants had detectable soluble Aβ as measured by 4G8. 13C3 and 4G8 were highly correlated ($r = 0.66$, $P < 0.001$). Protofibrillar Aβ42 and soluble Aβ were correlated with plasma Aβ42 and Aβ40 at baseline and at the follow-up assessment. The strongest correlation was between 13C3 and Aβ42 at baseline and follow-up (0.20, $P < 0.001$ and 0.36, $P < 0.001$, respectively) (Table 4). The highest detectable levels of protofibrillar Aβ42 were present among individuals with the highest plasma Aβ42 levels

Table 2. Relation of initial Aβ peptide levels to incidence of AD

Aβ levels	No. at risk	AD, *n* (%)	Hazard rate, model A†	Hazard rate, model B‡
Quartile of Aβ 40				
9.0–34.9	281	21 (6.7)	1.0 (reference)	1.0 (reference)
35–73.35	281	31 (10.6)	1.3 (0.7–2.4)	1.3 (0.6–2.7)
72.5–113.2	282	25 (9.3)	1.0 (0.5–2.1)	1.0 (0.5–2.1)
113.6–588.1	279	27 (10.9)	1.4 (0.7–2.9)	0.9 (0.4–2.1)
Quartile of Aβ 42				
9.0–18.8	282	15 (5.1)	1.0 (reference)	1.0 (reference)
18.85–33.4	281	27 (9.2)	1.9 (0.9–3.8)	2.1 (0.9–4.6)
33.45–49.25	281	31 (11)	2.2 (1.1–4.7)*	2.3 (1.0–5.4)*
49.3–198.7	281	31 (12.1)	3.4 (1.6–7.6)**	3.5 (1.4–8.6)**
Quartiles of Aβ 42/Aβ 40 ratio				
0.07–0.3530	281	23 (8.2)	1.0 (reference)	1.0 (reference)
0.35–0.51	281	24 (8.5)	1.1 (0.6–1.9)	1.2 (0.6–2.2)
0.51–0.75	281	30 (10.7)	1.2 (0.7–2.0)	1.6 (0.8–2.9)
0.75–7.40	279	27 (9.6)	0.9 (0.5–1.7)	0.9 (0.5–1.7)

*, $P < 0.05$; **, $P < 0.01$.
†Cox proportional hazards model and 95% confidence interval, with Aβ 42 and Aβ 40 in the model, unadjusted.
‡Cox proportional hazards model and 95% confidence interval, adjusted for age at baseline, cohort membership, sex, ethnicity, education, BMI, and the presence of the APOE ε 4 allele.

Table 3. Relation of change Aβ peptide levels to incidence of AD

Change in Aβ levels	No. at risk	AD, *n* (%)	Odds ratio, model A†	Odds ratio, model B‡
Change as a continuous variable				
Change in Aβ 40	1123	104 (9.3)	1.003 (0.99–1.01)	1.002 (0.99–1.01)
Change in Aβ 42	1125	104 (9.2)	0.98 (0.97–0.99)*	0.98 (0.97–0.99)*
Change in Aβ 42/Aβ 40 ratio	1123	104 (9.3)	0.77 (0.55–1.08)	0.7 (0.47–1.01)
Change in Aβ 40 by group				
Increasing	626	59 (9.4)	1.0 (reference)	1.0 (reference)
No change	434	41 (9.4)	0.9 (0.6–1.4)	0.7 (0.4–1.3)
Decreasing	58	4 (6.9)	0.6 (0.2–1.7)	0.5 (0.2–1.7)
Change in Aβ 42 by group				
Increasing	493	39 (7.9)	1.0 (reference)	1.0 (reference)
No change	502	41 (8.2)	1.1 (0.7–1.8)	1.5 (0.7–2.0)
Decreasing	130	24 (17.6)	2.8 (1.6–5.1)***	2.6 (1.3–5.1)**
Change in Aβ 42/Aβ 40 ratio by group				
Increasing	93	3 (2.9)	1.0 (reference)	1.0 (reference)
No change	692	65 (9.4)	3.1 (1.0–10.1)	3.2 (0.9–11)
Decreasing	333	36 (10.8)	3.6 (1.1–12.1)*	3.4 (1.0–11.8)*

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
†Logistic regression model, with Aβ 42 and Aβ 40 in the model, unadjusted.
‡Logistic regression model, adjusted for age at baseline, cohort membership, sex, ethnicity, education, BMI, and the presence of the APOE ε 4 allele.

(Table 4). Total soluble Aβ, as expected, correlated with both plasma Aβ40 and Aβ42 levels.

Compared with those who never developed cognitive or functional impairment throughout their participation in the study, levels of protofibrillar Aβ42 declined significantly over the follow-up in those who had developed mild AD by the second assessment (2.66 vs. −100.67, $P = 0.016$) (Table 5), but did not decline in those with prevalent mild AD (2.66 vs. −12.2, $P = 0.748$) (Table 5). Change in total soluble Aβ was not significantly different for those with either incident or prevalent mild AD compared with those who never developed mild AD, although total soluble Aβ tended to increase slightly with onset and duration of mild AD (Table 5).

Discussion

Compared with individuals with low plasma Aβ42 levels at baseline, those with high Aβ42 levels had more than a threefold increased risk of developing AD over an average of four 4.5 years. At the follow-up assessment when blood sampling was repeated, a decrease in plasma Aβ42 levels but not Aβ40 levels was related to the development of AD. The likelihood of having converted to AD 18–24 months before the second blood draw was three times higher when plasma Aβ42 levels had decreased by more than half of a standard deviation or when the plasma Aβ42/Aβ40 ratio decreased by more than half of a standard deviation. Thus, over time, decreasing levels of plasma Aβ42 or a decline in the Aβ42/Aβ40 ratio are sensitive indicators of recent conversion to AD. We postulate that the decline in plasma Aβ42 reflects compartmentalization of Aβ peptides in brain.

These results confirm and extend findings in nondemented individuals who subsequently developed late onset AD (11, 13, 14)

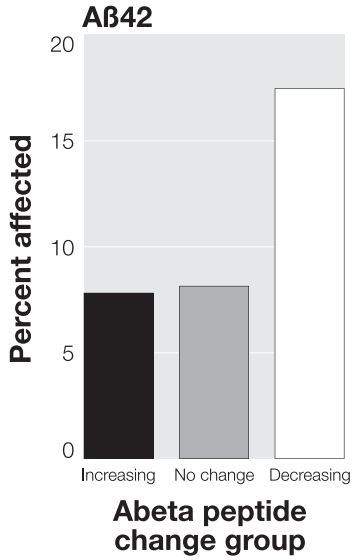

Fig. 1. Proportion of subjects with incident AD by Aβ42-change group.

Table 4. Relationship between Aβ 40 and Aβ 42 quartiles at baseline and follow-up and mean levels of antibodies to protofibrillar Aβ and total soluble Ab

Baseline		
Aβ 40 quartiles	R13C3	R4g8***
Lowest to 34.9	12.977	155.464
34.9–72.3	21.164	162.903
72.3–113.2	11.646	289.596
113.2 & higher	12.541	423.085
Aβ 42 quartiles	R13C3**	R4g8***
Lowest to 18.8	1.804	139.267
15.8–33.4	7.844	187.846
33.4–49.3	11.398	235.724
49.3 & higher	60.056	450.697
Follow-up		
Aβ 40 quartiles	R13C3	R4g8***
Lowest to 94.3	8.223	595.419
94.3–115.5	0.866	645.790
115.5–139.9	23.185	955.611
139.9 & higher	129.700	1142.849
Aβ 42 quartiles	R13C3	R4g8***
Lowest to 32.3	1.760	716.469
32.3–44.4	12.156	766.568
44.2–62.5	102.914	955.141
62.5 & higher	109.699	1165.050

Adjusted for age at blood draw. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 5. Relation of change in R13C3 and R4g8 to incident AD

	n	Change in R13C3, mean (SE)*	*P* value**	Change in R4g8, mean (SE)	*P* value**
No dementia	209	2.66 (24.2)	1.00		
Incident mild AD	91	−100.67 (34.3)	.016		
Prevalent mild AD	102	−12.20 (35.8)	.748		
No dementia	209			579.74 (42.2)	1.00
Incident mild AD	91			614.86 (59.82)	.637
Prevalent mild AD	102			653.68 (62.28	.647

*Adjusted for age, cohort membership, sex, ethnicity, education, APOE ε 4, and BMI. Incident and prevalent mild dementia vs. no dementia.

**Incident or prevalent mild AD compared with no dementia.

and are consistent with studies in women with mild cognitive impairment (10) and among asymptomatic first-degree relatives of patients with late onset AD (6), both groups at which are high risk of developing AD. Our results do not support prior studies that showed (*i*) no relation between plasma Aβ peptide levels and risk of AD (22); (*ii*) an association between low plasma Aβ40 and AD (23, 25); or (*iii*) a relation between a low plasma Aβ42/Aβ40 ratio and subsequent cognitive impairment and AD (24).

A number of factors may account for these inconsistencies with prior research. The most important factor is likely to be the timing of sample collection in relation to the preclinical period or to the stage of disease onset and progression. Few studies have examined risk associated with change in plasma Aβ peptide levels or change in Aβ42/Aβ40 ratio over time. In the current study, conversion to AD was strongly related to a decline in Aβ42 levels and in the Aβ42/Aβ40 ratio. Similarly, in a study of healthy nondemented elderly individuals, higher initial Aβ42 levels and greater reductions in Aβ42 levels over an ≈4-year period were associated with greater cognitive decline (15). In the CSF, low levels of Aβ42 and Aβ42/Aβ40 ratios in patients with mild cognitive impairment are associated with higher brain amyloid load (17, 18) and predict conversion to AD (16, 26). Our findings suggests that a decline in Aβ42 levels and in Aβ42/Aβ40 ratios can herald the onset of AD, possibly reflecting sequestration of Aβ42 in senile plaques or the formation of semisoluble oligomers (27, 28).

Formation of protofibrillar forms of Aβ42 has been suggested to be the initial toxic event leading to onset of AD. Neurotoxicity is associated with several self-associating Aβ assemblies, including subfibrillar Aβ-derived diffusible ligands (ADDLs) and protofibrils (29). In rats *in vivo* or in primary neuronal cultures, ADDLs are associated with inhibition of hippocampal long-term potentiation (28, 30). Brain and CSF levels of ADDLs are elevated in AD brains (31–33), and formation of protofibrillar forms of Aβ is found in association with high levels of β-amyloid in transgenic mice (7, 29, 34). We used a novel antibody to examine the relation of a protofibrillar form of Aβ42 in plasma to onset of an early, mild stage of AD. We hypothesized that protofibrillar forms of Aβ would decline in plasma as cognitive impairment developed and that total soluble Aβ would increase in plasma with age. Among those with prevalent mild AD at baseline, we hypothesized that change in protofibrillar Aβ42 would not differ from those who never developed dementia, because the protofibrillar Aβ42 associated with the development of AD would have already gained entry into the brain. Our observations that the highest detectable levels of protofibrillar Aβ42 were present among individuals with the highest plasma Aβ42 and Aβ40 levels and that decline in protofibrillar Aβ42 but not total soluble Aβ was associated with conversion to mild AD support this conclusion. It is noteworthy that protofibrillar Aβ42 was found in only a minority of participants (34%), suggesting that variation in plasma levels of protofibrillar Aβ may be a biomarker of change in cognitive status and explain some of the variability in risk seen among participants with high levels of Aβ42 at baseline when the presence of protofibrillar forms is not considered.

A limitation of this study is that measures of protofibrillar Aβ42 were available for only a subset of the sample. Plasma Aβ42 and Aβ40 levels have been suggested as potential biomarkers for the development of late onset AD. Our results support a model in which the decline in these Aβ species likely reflects compartmentalization of Aβ peptides in brain with onset of dementia and with protofibrillar species most highly correlated with onset of cognitive impairment. Further work is needed on the pattern of change in Aβ levels associated with the development of AD and how that pattern may vary between familial and sporadic AD or by the presence of genetic or environmental risk factors.

Materials and Methods

Study Population. Plasma Aβ40 and Aβ42 and clinical data were obtained from participants in the Washington Heights-Inwood Columbia Aging Project, a prospective study of aging and dementia among Medicare recipients 65 years and older residing in northern Manhattan. Subjects were recruited in two waves, one ending in 1992 and the other in 1999. The sampling strategies and recruitment outcomes of these two cohorts have been described in detail elsewhere (35). The cohort used for the current study represents a combination of continuing members of the cohort originally recruited in 1992 ($n = 602$) and members of a new cohort recruited between 1999 and 2001 ($n = 2,174$). The population from which participants were drawn was comprised of individuals from several different countries of origin representing three broadly defined ethnic categories (i.e., Caribbean Hispanic, African-American, and non-Hispanic White of European ancestry). Individuals who completed a baseline and second follow-up assessment and consented to provide a blood sample were included in the study ($n = 1,125$). The Columbia University Institutional Review Board reviewed and approved this project. All individuals provided written informed consent.

Clinical Assessments. Each participant underwent an in-person structured interview of health and functional ability, followed by a standardized assessment, including medical and medication history, physical and neurological examination, and a comprehensive neuropsychological test battery (36) at the time of study entry, which was repeated at ≈18- to 24-month intervals. Stroke was defined according to the World Health Organization criteria, based on self-report, and supplemented by a neurological examination. Diabetes, hypertension, heart disease, and history of other medical conditions were ascertained by self-report at each visit. By using a standard protocol, standing body weight to the nearest 0.1 kg, measured with a balance scale (Scale-Tronix), and height without shoes to the nearest 0.5 cm, measured by using an anthropometer (GPM), were used to calculate the BMI (kg weight/m^2 height).

Cognitive Assessment. The neuropsychological test battery, previously validated for this geographical area (36, 37), was administered in either Spanish or English. The battery consisted of the orientation subtest from the modified MiniMental State Examination (38), the Boston Naming Test (39), the Controlled Word Association test (40), category naming, the Complex Ideational Material and Phrase Repetition Subtests of the Boston Diagnostic Aphasia Evaluation (41), the Abstract Reasoning and Similarities subtests from the

Wechsler Adult Intelligence Scale-Revised (42), the nonverbal Identities and Oddities subtest of the Mattis Dementia Rating Scale (43), the Rosen Drawing Test (44), the matching version and the multiple-choice version of the Benton Visual Retention Test (45), and the Selective Reminding Test (46).

Diagnosis of Dementia. The diagnosis of dementia was based on standard research criteria and was established by using all available information gathered from the initial and follow-up assessments and medical records by consensus at a conference of physicians, neurologists, neuropsychologists, and psychiatrists. AD diagnosis was based on National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria for probable and possible AD (47). Severity of AD was rated by using the Clinical Dementia Rating Scale (48). Only participants with a diagnosis of probable or possible AD were included in the analysis. Participants showing a slight decline in cognitive performance and impairment in daily activities but who were still living independently were considered to have mild AD (48).

Ethnic Group. At baseline, ethnic group was documented by self-report using the format of the United States Census (49). Each individual was first asked to indicate his racial group and then whether or not he was of ''Hispanic origin.''

Apolipoprotein (APOE) Genotype. Genotypes were obtained by amplification of genomic DNA with PCR subjected to *Cfo*I restriction analysis using APOE primers and conditions similar to those described by Hixson and Vernier (50) and modified by Maestre *et al.* (51). Participants were classified according to the presence or absence of an APOE ε4 allele.

Plasma Aβ40 and Aβ42. A 10-ml sample of venous blood (K_3EDTA lavender-top tubes) was collected at baseline and the second follow-up for plasma Aβ peptide levels. Plasma levels of Aβ42 and Aβ40 were measured blind to dementia status by using a combination of monoclonal antibody 6E10 (specific to an epitope present on 1–16 amino acid residues of Aβ) and rabbit antisera R165 (vs. Aβ42) and R162 (vs. Aβ40) in a double-antibody sandwich ELISA as described previously (13, 21). The detection limit for these assays was 9 pg/ml for Aβ40 and 10 pg/ml for Aβ42. Aβ peptide levels from each sample were measured twice, using separate aliquots so that none of the samples were refrozen and rethawed for the repeat assay. Previously, we had established that the test-retest reliability of the measurement of plasma Aβ40 and Aβ42 was excellent (Cronbach's α coefficient = 0.91). There were 1,270 participants for whom blood samples were available; we were able to obtain Aβ peptide levels for 1,242 (97.8%) participants. Among these, 64 samples were at the lower limit of detection for Aβ40 (5.8%) and 100 samples were at the lower limit of detection for Aβ42 (8.9%).

Monoclonal Antibody Against Protofibrillar Aβ, Clone 13C3. NaOH-treated synthetic Aβ42 peptides (American Peptide) were polymerized to form fibrilliar protein structures, and the degree of fibrillar Aβ42 aggregation was monitored by circular dichroism spectroscopy as described previously (52). BALB/c *fcgr2* deficient mice were immunized with the fibrillar form of the Aβ42 protein, and hybridomas were generated in the Monoclonal Antibody Core Facility at the Memorial Sloan–Kettering Cancer Center by using standard methods. The hybridoma supernatants were screened by ELISA for high-affinity monoclonal antibodies against the fibrillar form of the Aβ42 protein and were also scored for specificity by immunohistochemistry on murine brain sections for amyloid burden. After separating protofibrillar Aβ42 and low molecular weight (LMW) Aβ42 by the method described previously (53) [supporting information (SI) Fig. S1], monoclonal antibodies specific to the protofibrillar Aβ42 protein were evaluated by surface plasmon resonance (Biacore, GE Healthcare) (Fig. S2) and by ELISA (Fig. S3). Two hybridoma clones reacted with the protofibrillar Aβ42 fractions but not with the LMW fractions. Clone 13C3 showed the highest affinity and was later used for immunoassays to measure plasma Aβ42 levels in patients.

Statistical Analyses. Analyses were restricted to participants who were not demented at baseline, excluding 104 participants with prevalent AD. An additional 13 individuals classified as having ''other'' ethnicity were also excluded, leaving 1,125 participants for the analysis. In preliminary analyses, we used χ^2 tests for categorical variables and Student's *t* test and ANOVA for continuous variables to compare nondemented and incident cases of AD by demographic characteristics and levels of Aβ peptides. Two sets of analyses were conducted. First, we examined the relation of Aβ peptides at baseline to risk of incident AD. We used Cox proportional hazards modeling to estimate the cumulative incidence and HR of AD by quartile of Aβ peptide. The time-to-event variable was time from baseline to onset of AD for incident cases and time from baseline to last assessment for those who remained nondemented. Second, we examined the relation of change in Aβ peptides to risk of AD. Change was calculated as the difference between levels at the second follow-up minus the level at baseline. We examined change both as a continuous variable and by change groups. We classified changes in Aβ peptides from baseline to follow-up into three groups based on 0.5 standard deviations of change: (*i*) no change (no change $\pm$ 0.5 SD of change), (*ii*) increasing ($>$0.5 SD of change), and (*iii*) decreasing ($<$0.5 SD of change). We used logistic regression models to estimate the likelihood of AD by change in level of Aβ peptides or by change in Aβ peptide group, with increasing levels as the reference group. Because levels of Aβ42 and Aβ40 were correlated, we used models containing measures of both peptides in all analyses to determine if independent relationships with dementia status were present. All analyses were conducted first in univariate models (model A) including both Aβ42 and Aβ40 and then in models that adjusted for age, cohort membership, sex, ethnic group, education, BMI, and the presence of the APOE ε4 allele (Model B).

We also examined mild AD, characterized by cognitive impairment with mild functional deficits as an outcome when determining the effects of protofibrillar Aβ42 and soluble Aβ. Participants were classified as having no mild AD, prevalent mild AD, or incident mild AD. Mild AD was used as the outcome to provide an index of the earliest changes associated with onset of AD. We used multivariate ANOVA to assess the relation of changes in levels of 13C3 and 4G8 antibodies to incident and prevalent mild AD, adjusting for age, cohort membership, sex, ethnic group, education, BMI, and the presence of the APOE ε4 allele.

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