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Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study

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Abstract

Objectives—To examine the association between incident Alzheimer disease (AD), and plasma Aβ1-40 and Aβ1-42 levels in normal and mild cognitive impairment (MCI) subjects in a subgroup of participants of the Cardiovascular Health Study Cognition Study.

Methods—We determined the plasma Aβ1-40 and Aβ1-42 levels of 274 nondemented subjects (232 normals and 42 with MCI) in 1998 –1999 and repeated the measurements in 2002–2003. The mean age of the subjects at baseline was 79.3 ± 3.6 years. We examined the association between Aβ levels and incident AD over the ensuing 4.5 years, controlling for age, cystatin C level (marker of glomerular function), apolipoprotein E-4 allele, Modified-Mini-Mental State Examination scores, and MRI-identified infarcts.

Results—In an unadjusted prospective model in normal subjects, both Aβ1-40 and Aβ1-42 levels in 1998 –1999 were associated with incident AD (n = 55) in 2002–2003 (longitudinal analysis). In the fully adjusted multivariate model, neither Aβ1-42 nor Aβ1-40 nor their ratio was associated with incident AD. However, adjustment had a very small effect on point estimates for Aβ1-42, from an odds ratio (OR) of 1.61 (p = 0.007) in the unadjusted model to an OR of 1.46 (p = 0.08) in the fully adjusted model. In 2002–2003 (cross-sectional analysis), only the unadjusted models showed that both peptides were associated with AD.

Conclusions—Plasma Aβ levels are affected by age and by systemic and CNS vascular risk factors. After controlling for these conditions, Aβ-40 and Aβ1-42 are weak predictors of conversion to Alzheimer disease (AD) in normal subjects and are only weakly associated with AD in cross-sectional analysis. Consequently, plasma levels of Aβ do not seem to be useful biomarkers for AD.

There is growing evidence that aggregation and accumulation of Aβ proteins are the central pathologic processes associated with neurodegeneration in patients with Alzheimer disease (AD).[1] Aβ proteins are proteolytically derived from the β amyloid precursor protein, and the most important components of the senile plaque are Aβ1-42 and Aβ1-40; Aβ1-42 is deposited first, and it is the predominant form in the senile plaque, whereas the Aβ1-40 is deposited later.[2,3] The concentrations of Aβ1-42 are decreased in AD patients, whereas Aβ1-40 levels remain normal in the CSF,[4,5] possibly because Aβ1-42 is deposited faster than Aβ1-40 in the brain.

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Therefore, the ratio of Aβ1-42 to Aβ1-40 may better discriminate AD patients from normals than CSF Aβ1-42 alone.[4] However, these data are based on cross-sectional studies—longitudinal studies have shown a great variability in CSF Aβ levels; some have shown Aβ1-42 decrease over time,[6] whereas others have shown an increase.[7]

Aβ amyloid proteins can also be found in the plasma, and are increased in both familial and sporadic forms of AD.[8] Cross-sectional studies have shown that plasma β amyloid levels are elevated in AD.[9,10] The reason for the discrepancy between low CSF and high plasma Aβ1-42 in AD has not been resolved, but it may be because plasma levels of Aβ amyloid proteins can be modulated by multiple factors. Plasma Aβ amyloid levels increase with age[11]; are higher in subjects with cerebrovascular disease and white matter lesions, especially Aβ1-40[12–14]; and are influenced by renal function.[15] Plasma Aβ levels are also significant predictors of death.[10]

Plasma Aβ1-42 and Aβ1-40 are increased in nondemented subjects who subsequently progress to AD.[10,16,17] Increased levels of plasma Aβ1-40 and a low Aβ1-42/Aβ1-40 ratio were predictors of AD in normal individuals, but not of vascular dementia,[17] but it has also been shown that neither Aβ1-40 nor Aβ1-42 was a predictor of AD, and that only low Aβ1-42/Aβ1-40 ratio was predictive,[18] suggesting that Aβ1-42 levels diminish before AD is clinically evident, which would mirror what is seen in the CSF levels.[18] However, this latter study combined patients with AD or mild cognitive impairment (MCI; an intermediate state between normalcy and dementia) into one group of incident cognitive impairment, independent of severity.

Because Aβ amyloid deposition may be an essential early event in the AD pathologic process, it has been hypothesized that plasma Aβ1-42 and Aβ1-40 levels should increase before the onset of the symptoms and decline as the disease progresses. However, there are few studies with serial plasma amyloid measurements that could test this hypothesis. Mayeux et al.[10] found that Aβ1-40 tended to remain stable or increased in prevalent and incident AD cases, whereas the Aβ1-42 levels declined at a rate of 1.006 pg/mL per year. However, there was a high mortality rate in this cohort, and only 58% of the initial cases had a second plasma Aβ amyloid assessment.

The purpose of the present study was to examine the plasma Aβ amyloid levels of a group of normal and MCI subjects from a large longitudinal epidemiologic study. Subjects had plasma amyloid levels in 1998–1999 and repeat measurements in 2002–2003. We tested the hypothesis that both Aβ1-42 and Aβ1-40 are increased before the onset of dementia in normal subjects and that they will remain elevated after the development of clinical AD.

METHODS

The Pittsburgh Cardiovascular Health Study Cognition Study (CHS-CS) began in 1992–94, at the time that the participants completed an initial MRI of the brain, and in 2002–2003 we conducted a study to determine the incidence of dementia and MCI in a population of normal and MCI subjects identified in 1998–1999 in the CHS-CS.[19] Of the 924 participants examined in 1992–1994, a total of 532 normal and MCI subjects were available for study in 1998–1999. These subjects had annual cognitive tests from 1989–1990 to 1998–1999 (see below) and complete neurologic and neuropsychological examinations in 1998–1999 and 2002–2003.[19,20] In addition to the MRI of the brain obtained in 1992–1994, a second MRI was performed in 1998–1999, and 157 participants had also an MRI in 2002–2003. The MRI of the brain in 2002–2003 was obtained when a participant changed diagnosis from normal to MCI, from MCI to dementia, or from normal to dementia. The characteristics of the total Cardiovascular

Health Study (CHS) cohort and the Pittsburgh CHS-CS have been described previously,[21] as well as the details regarding the longitudinal follow-up of the CHS participants.[21,22]

Longitudinal data

Beginning in 1989–1990, all participants in the CHS completed the Modified Mini-Mental State Examination (3MSE)[23] and the Digit Symbol Substitution Test[24] at their annual visits, and the Benton Visual Retention Test from 1994 to 1999.[24] Further information on cognition was obtained from proxies using the Informant Questionnaire for Cognitive Decline in the Elderly[25] and the Dementia Questionnaire.[26] Symptoms of depression were measured annually with the modified version of the Center for Epidemiology Studies Depression Scale (CES-D).[27] Data on instrumental activities of daily living (IADLs) and activities of daily living were also collected at every clinic visit.[21]

Subjects

Subjects were included in this analysis if they were alive at both time points, had blood samples available in both 1998–1999 and 2002–2003, and were classified according to the CHS cognitive criteria.[21,22] The characteristics of the 274 participants who were eligible for this study and those who were not are shown in table e-1 on the *Neurology*® Web site at www.neurology.org. The participants who entered this study were younger, had better 3MSE scores, and had lower cystatin C levels[28] than those who did not enter the study.

Clinical examination in 1998–1999 and 2002–2003

The neurologic examination included a brief mental status examination, as well as cranial nerve testing, motor tone, abnormal movements, strength, deep tendon reflexes, release signs, plantar response and clonus, cerebellar testing, primary sensory testing, gait, and postural stability. The examiner also completed semistructured examinations for parkinsonism and interviews for cerebrovascular disease.[19,20] Symptoms of depression were measured with the modified version of the CES-D 10-item version, and additional behavioral symptoms were measured with the Neuropsychiatric Inventory.[29] All participants were assessed with cognitive tests that examined the following cognitive domains: pre-morbid intelligence, verbal and nonverbal memory, language, visuoperceptual and visuoconstructional, psychomotor speed, executive functions, and fine motor control. Details of the neuropsychological battery have been published previously.[30]

MCI criteria

MCI subjects were diagnosed following the CHS-CS diagnostic criteria for two MCI subgroups.[20] The MCI–amnestic type included subjects with impairments (defined as performance > 1.5 SD below age-/education-appropriate means) in delayed recall of verbal material, nonverbal materials, or both, and the cognitive deficits must represent a decline from a previous level of functioning. The other cognitive functions must otherwise fall within normal limits. The second type, MCI–multiple cognitive deficits type, required impairments in at least one cognitive domain other than memory (i.e., two or more tests abnormal) or one abnormal test (which could be a memory test) in at least two separate domains, without sufficient severity or loss of IADLs to constitute dementia. These cognitive deficits may or may not affect IADLs but must represent a decline from a previous level of functioning as reported by the proxy or the participant.

Dementia diagnosis

The diagnosis of dementia was based on a deficit in performance in two or more cognitive domains that were of sufficient severity to affect the subjects' activities of daily living, and history of normal intellectual function before the onset of cognitive abnormalities. An abnormal

domain was present when at least two tests of the same domain were abnormal. These criteria have been used successfully over the past 20 years, and they have shown a sensitivity of 98% and a specificity of 88% for AD.[31] All subjects with the diagnosis of dementia in this study met the criteria for possible or probable AD.[32]

Plasma amyloid measurements

The samples used for the assays—both samples collected in 1998–1999 and 2002–2003—were defrosted in 2006 and measured at the same time using the same methodology. The general blood collection and laboratory methods in CHS have been published previously. [33]

Aβ1-40 and Aβ1-42 antisera

Aβ32-40 and Aβ33-42 peptides synthesized commercially (Ana Spec, San Jose, CA) were conjugated to keyhole limpet hemocyanin in phosphate-buffered saline (PBS) with 0.5% glutaraldehyde, and immunized in rabbits. The specificity of rabbit antisera produced against Aβ1-40 and Aβ1-42 was examined in a sandwich ELISA. There was a strong response of Aβ1-40–specific antibody with 1 ng/mL of Aβ1-40 but no detectable response with 10 ng/mL of Aβ1-42. Similarly, Aβ1-42 antibody was found to be specific to Aβ1-42 but showed no reactivity to Aβ1-40. Western blot also showed that antibodies to Aβ1-40 and Aβ1-42 were specific for each of these proteins. The method used here assessed free circulating plasma Aβ levels. However, it has been established that Aβ binds to a number of blood proteins (e.g., apolipoproteins, α 1-antichymotrypsin, cytokines), which is difficult to quantify. [34]

Aβ ELISA

Aβ1-40 and Aβ1-42 levels were measured in plasma using a combination of mouse monoclonal antibody 6E10 (specific to an epitope present on 3–16 amino acid residues of Aβ) and two different antibodies specific for Aβ1-40 and Aβ1-42, in a double antibody sandwich ELISA. Briefly, wells of microtiter plates were coated with 100 μL of 6E10 (2.5 μg/mL) diluted in carbonate–bicarbonate buffer, pH 9.6, and incubated at 4°C overnight. After washing the plates with PBS containing 0.05% of Tween-20 (PSBT), plates were blocked for an hour with 1% bovine serum albumin (BSA) in PBST to avoid nonspecific binding. The plates were washed, and 100 μL of Aβ1-40 or Aβ1-42 peptides standard ranging from 625 pg/mL to 5 pg/mL diluted in PBST + 1% BSA or undiluted plasma, was applied and incubated for 2 hours at room temperature and 4°C overnight. After washing, plates were incubated with 100 μL of appropriately diluted biotinylated Aβ1-40 or Aβ1-42 antibodies and were allowed to stand for 1 hour 30 minutes at room temperature. After washing, 100 μL of NeutrAvidin horseradish peroxidase conjugate (Pierce, Rockford, IL), diluted 1:10,000 in PBST, was added and incubated for 1 hour at room temperature. Plates were washed again, and 100 μL of 3,3′,5,5′-tetramethylbenzidine substrate solution (Kirkgaard and Perry Laboratories, Gaithersburg, MD) was added to each well. The reaction was stopped by adding 100 μL of 1 M phosphoric acid. The optical density (OD) was measured at 450 nm in a micro ELISA reader. The concentrations of Aβ1-40 and Aβ1-42 in the samples were calculated from the standard curve for each plate. The relationships between OD and Aβ peptide concentrations were determined by a four-parameter logistic log function. Nonlinear curve fitting was performed with the KinetiCalc program (Bio-tek Instruments, Inc. Winooski, VT) to convert OD of plasma to estimated concentrations. The detection limit for this assay was 10 pg/mL for Aβ1-40 and for Aβ1-42. The mean of the coefficient of variation within assay was 4.6% for Aβ1-40 and 9.3% for Aβ1-42.

Statistical analysis

Univariate analyses of the association between dementia status and plasma Aβ1-40 and Aβ1-42 and covariates were evaluated by *t* tests and χ^2 tests. The covariates included in the

analyses were age, 3MSE scores, APOE-4 allele, cystatin C,[28] and MRI-identified infarcts. Pearson correlation coefficients were calculated to examine the relation between plasma Aβ1-40 and Aβ1-42 and covariates. The associations between plasma Aβ1-40 and Aβ1-42 and incident dementia, compared with subjects who did not develop AD, were estimated using logistic regression analyses. The models were estimated first without any covariate adjustment, and then controlling for age, presence of the APOE-4 allele,[35] cystatin C levels (an estimate of glomerular excretion function),[28] 3MSE score, and MRI-identified infarcts. Because the distributions of Aβ measures were skewed, the levels were transformed ($\log_{10}$) before analysis. Odds ratios (ORs) were estimated with increments of 1 SD of plasma Aβ1-40 and Aβ1-42. Statistical analyses were performed with SPSS version 13. All analyses were two-sided at $\alpha = 0.05$. The small number of participant who remained as MCI in 2002–2003 precluded the multivariate analyses in the MCI group. Nevertheless, we provide complete clinical and laboratory data of these participants.

RESULTS

The baseline demographic and clinical characteristics of the normal and MCI participants are shown in table e-2. MCI subjects were more likely to be nonwhite, had lower education levels, had lower 3MSE scores, and had higher Aβ1-40 levels in 1998–1999 and 2002–2003 than normals. No statistical differences were noted in terms of age, sex, proportion of cases carrying the APOE-4 allele, and cystatin C levels, proportion of cases with MRI infarcts, and Aβ1-42 levels in 1998–1999 and 2002–2003.

Table 1 shows the demographic and clinical characteristics in 1998–1999 of the participants by diagnosis change in 2002–2003. Each group was compared and contrasted with those participants who remained normal from 1998 –1999 to 2002–2003. The subjects who converted from normal to AD were older ($t = -2.75$, $p < 0.001$), had lower 3MSE scores ($t = 4.11$, $p < 0.001$), had higher cystatin C levels ($t = -2.09$, $p = 0.03$), had more MRI-identified infarcts ($\chi^2 = 6.98$, $p = 0.008$), and had higher Aβ1-40 (in 1998–1999: $t = -1.92$, $p = 0.05$; and in 2002–2003: $t = -2.17$, $p = 0.03$), Aβ1-42 (in 1998–1999: $t = -2.92$, $p = 0.004$; and in 2002–2003: $t = -2.28$, $p = 0.02$), and Aβ1-42/Aβ1-40 ratio (only in 1998 –1999: $t = -2.15$, $p = 0.03$) than those who remained normal. The normal subjects who progressed to MCI had lower 3MSE scores ($t = 3.36$, $p = 0.001$) compared with those who remained normal. The subjects who converted from MCI to AD were less likely to be white ($\chi^2 = 12.4$, $p = 0.001$), had lower education levels ($\chi^2 = 10.4$, $p = 0.001$), had lower 3MSE scores ($t = 10.2$, $p < 0.001$), had higher cystatin C levels ($t = -2.07$, $p = 0.04$), and had higher Aβ1-40 levels in 1998 –1999 ($t = -2.34$, $p = 0.02$) and 2002–2003 ($t = -2.61$, $p = 0.01$) than those who remained normal. The MCI participants who remained stable were less likely to be white ($\chi^2 = 5.79$, $p < 0.01$), had lower education levels ($\chi^2 = 7.46$, $p < 0.01$), and had lower 3MSE scores ($t = 8.50$, $p < 001$) than those who remained normal. Finally, the Aβ levels between groups were subsequently analyzed with a univariate analysis of variance controlled for age in the groups where we detected a statistically significant difference in the *t* test; the differences remained unchanged.

The Aβ1-40 and Aβ1-42 levels increased in all groups from 1998 –1999 to 2002–2003 (mean follow-up 4.5 years), except for the Aβ1-42 level in the MCI group, which remained stable. The mean β1-40 change was 20.1 pg/mL in the participants who remained normal, 31.6 pg/mL in the normals who progressed to AD, 25.4 pg/mL in the normals who progressed to MCI, 33.4 pg/mL in the MCI who progressed to dementia, and 21.6 pg/mL in the MCI stable group. The mean β1-42 change was 4.9 pg/mL in the participants who remained normal, 7.0 pg/mL in the normals who progressed to AD, 7.2 pg/mL in the normals who progressed to MCI, and 2.7 pg/mL in the MCI subjects who progressed to dementia. By contrast, the Aβ1-42 level decreased 1.2 pg/mL in the stable MCI group.

Logistic regression analysis

The baseline plasma Aβ1-40 and Aβ1-42 levels were correlated with the variables entered in the logistic regression analysis (table e-3). Aβ1-40 and Aβ1-42 correlated with increased age and with cystatin C levels. There was a negative correlation between 3MSE scores with Aβ1-40 and Aβ1-42 levels. The Aβ1-42/Aβ1-40 ratio correlated with age and with Aβ1-40 and Aβ1-42 levels. There was a positive correlation among Aβ1-40 and Aβ1-42 levels, and the Aβ1-42/Aβ1-40 ratio.

The unadjusted and adjusted ORs for a 1-SD increase in β amyloid protein levels are shown in tables 2–4. In the unadjusted model (Model 1 in table 2), the Aβ1-40 and Aβ1-42 levels and Aβ1-42/Aβ1-40 ratio in 1998 –1999 were associated with incident AD in normal subjects (table 2). In Model 2, controlling for age, only Aβ1-42 level was associated with incident AD. Models 3 and 4 included measures of renal function (cystatin C) and cerebrovascular disease (MRI infarcts), and the association between Aβ1-42 levels and AD was no longer significant. However, the changes in the ORs were small, and the 95% CI overlapped. The Aβ1-42/Aβ1-40 ratio was also a significant predictor of incident AD in the unadjusted models (higher Aβ1-42 as compared with Aβ1-40), but not in the multivariate models.

With regard to the development of MCI among normal subjects, a slightly different pattern emerges (table 3). Aβ1-40 levels, but not Aβ1-42, were associated with developing MCI in normal subjects. Further, the OR became higher as the models adjusted for more covariates, although the 95% CIs overlapped in all cases.

Finally, we examined the association between Aβ levels and AD diagnosis in 2002–2003 (cross-sectional). In the unadjusted model, both Aβ1-42 and Aβ1-40, but not the ratio, were associated with the diagnosis of AD. However, when relevant confounders were accounted for, the effect became nonsignificant, although the 95% CIs continued to overlap (table 4).

There was a significant interaction between the presence of peripheral vascular disease (as indexed by cystatin C level) and Aβ1-42 (Wald = 3.83, $p = 0.05$), but not Aβ1-40 (Wald = 0.17, $p = 0.67$). This means that as the level of cystatin C increased, the level of Aβ1-42 also increased in the context of incident AD. The same was not true for the presence of MRI-identified infarcts. For neither Aβ1-42 nor Aβ1-40 was there a significant interaction with infarcts and incident AD.

DISCUSSION

In unadjusted models, we found that Aβ1-42 and Aβ1-40 were associated with conversion from normal cognition to dementia. However, after adjusting the models for relevant covariates—age, kidney function, cerebrovascular disease, genetic load (i.e., APOE-4 allele), and cognitive state—there was no significant association between plasma amyloid and incident AD. Therefore, Aβ plasma levels seem to be weak predictors of conversion to AD in cognitively normal individuals

Based on the results of this study, and a review of the work of others, it seems that there are three factors that could result in an elevation of the levels of Aβ1-42 and Aβ1-40. The first is advanced AD–related pathology. There are increased Aβ levels in plasma when AD pathology is present. However, two additional factors render this observation less important. First, plasma amyloid levels increase naturally with age. In the present study, the effects of amyloid levels on risk for AD were severely attenuated when we adjusted the model for the age of the participants. Fukumoto et al.[11] also found that levels of Aβ1-42 and Aβ1-40 increased with age, and after adjusting for age, they did not find differences between normal subjects and those with MCI, AD, or Parkinson disease. Second, there is an association between plasma

amyloid levels and cerebrovascular disease.[13,14,36] Further, peripheral vascular disease can also affect amyloid levels.

Plasma Aβ is excreted through the kidneys, so if renal excretion functions are decreased, plasma amyloid levels will increase, although this will be an artifact of renal dysfunction and not overproduction of amyloid in the brain.[15] We assessed kidney function with cystatin C, which is a better marker of glomerular excretion function than creatinine.[37] When we adjusted our models for cystatin C, this weakened further the associations between amyloid and incident AD. Disorders of the peripheral vasculature are common in the elderly and are frequently associated with hypertension and diabetes mellitus, both of which are also prevalent in older cohorts such as the CHS-CS. Thus, these two common age-related disorders can affect the peripheral vasculature with a resulting increase in Aβ1-42 and Aβ1-40. Furthermore, because both of these conditions are also associated with cerebrovascular disease,[38] this could result in an additive effect on plasma amyloid levels. These findings may explain the relationship between reduced insulin clearance and plasma β amyloid levels in AD patients,[39] and the modulation of plasma β amyloid levels by drugs (e.g., nonsteroidal anti-inflammatory drugs) that affect glomerular function in normal subjects.[40]

Thus, of the three factors that seem to affect the level of Aβ1-42 and Aβ1-40 in plasma, the bulk of the associations seem linked to age- and age-related diseases and disorders affecting the vasculature; an effect of an underlying AD-related pathology seems to be weak at best. How then can we reconcile these data with those from other studies? First, there is little by way of consensus among other studies as to the relationships among plasma amyloid, AD, and the development of clinical dementia. Some studies found that β1-42 levels in normal subjects were predictors of AD,[10,16] others β1-40 levels,[17] and others found no association for either peptide.[18] In addition, some studies have found high[10,16] or low[17,18] β1-42/β1-40 ratios in subjects who converted to AD. Furthermore, it seems that differences in cohort characteristics, methods of case ascertainment, whether the study is cross-sectional or longitudinal, and the timing of the measurements in terms of the natural history of dementia all affect whether an association will be found between plasma amyloid levels and AD. However, what may, perhaps, be the most important study characteristic is the case ascertainment. Determining whether a participant is cognitively normal can be difficult, and relying on screening tests may be problematic. The mean 3MSE score of our impaired subjects was 85.1, which is well above the traditional cutoff of 78 to 80 used in many epidemiologic studies.[41] Thus, relying solely on a screening test (or a small test battery) could result in a contamination of the “normal” group with subjects with very mild dementia (or MCI).[17] The best studies, therefore, would be those with both adequate evaluation of cognitive functions *and* sufficient longitudinal data to ensure that subjects are classified as well as possible.

We did find an association between incident MCI and elevations of Aβ1-40 in the fully adjusted model. There are several possible explanations for this finding. MCI, for the purposes of this study, included individuals with possible etiologies other than AD, including systemic, neurologic, and psychiatric, and it is possible the vascular factors may be more important here. However, there is a more interesting possibility related to the characteristics of MCI itself. The incident AD cases developed their dementia in a relatively short period of time, 4 to 5 years.[19] As the subjects progressed from cognitive normality to dementia, they surely passed through an MCI phase, albeit briefly. Consequently, it is possible that if we had obtained blood samples on an annual basis, we could have detected the critical time when plasma amyloid was significantly elevated. However, for the subjects who progressed from normal cognition to MCI over the same time period, the transition to dementia may be moving more slowly, providing us with the opportunity to sample during the transition phase and perhaps see an increase in plasma amyloid. At this point, we do not know whether that increase was associated with a developing AD, or new vascular changes that could also increase amyloid levels.

However, the fact that Aβ1-40 was elevated in the MCI subjects who transitioned to dementia suggests that at least some of the amyloid was due to the AD process. Thus, there may be a brief window, during the MCI phase, when measurement of plasma amyloid may be useful.

Finally, AD pathology, age, and age-related diseases and disorders affecting the vasculature have a significant impact on amyloid levels and attenuate the importance of the amyloid assay. However, if an individual is in a slowly evolving dementia syndrome, there may be a window of opportunity where an elevation of plasma Aβ indicates that AD is an important component of the MCI syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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GLOSSARY

3MSE
: Modified Mini-Mental State Examination

AD
: Alzheimer disease

BSA
: bovine serum albumin

CES-D
: Center for Epidemiology Studies Depression Scale

CHS
: Cardiovascular Health Study

CHSCS
: Cardiovascular Health Study Cognition Study

IADL
: instrumental activities of daily living

MCI
: mild cognitive impairment

OD
: optical density

OR
: odds ratio

PBS
: phosphate-buffered saline

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Table 1

Baseline clinical characteristics according to diagnosis change to 2002–2003, and Aβ levels in 1998–1999 and 2002–2003 in normal and MCI subjects

		Each subgroup was compared with those who remained normal			
	Remained normal	**Normal to dementia**	**Normal to MCI**	**MCI to dementia**	**MCI stable**
No. of subjects	117	55	60	33	9
Age, y	78.6 ± 3.6	80.2 ± 3.6‡	79.5 ± 2.8	79.6 ± 4.3	80.5 ± 4.3
White	93 (79.5)	43 (78)	50 (83)	16 (48.5)‡	4 (44)†
> High school education	79 (67.5)	30 (54)	40 (67)	12 (36)‡	6 (78)†
Male/female	49/68	16/39	22/38	17/16	3/6
3MSE	97.4 ± 2.7	94.9 ± 5.3†	95.6 ± 4.3‡	89.2 ± 7.0‡	88.7 ± 7.3‡
APOE-4 allele, n = 253	21 (20)	16 (31)	9 (16)	11 (37)	1 (11)
Cystatin C, mg/L	0.98 ± 0.17	1.05 ± 0.24*	0.99 ± 0.18	1.07 ± 0.36*	0.94 ± 0.18
MRI infarcts	21 (18)	20 (36)†	13 (22)	4 (21)	2 (22)
Hypertension§	48 (42)	22 (41)	24 (41)	17 (51)	2 (22)
Diabetes mellitus¶	11 (10)	8 (15)	11 (19)	6 (18)	2 (22)
Heart disease‖	19 (16)	6 (11)	9 (15)	5 (15)	2 (22)
Plasma Aβ-40					
Aβ-40 in 1998–1999	142.1 ± 59.7	161.4 ± 55.6*	154.2 ± 48.3	173.7 ± 87.5*	166.9 ± 42.5
Aβ-40 in 2002–2003	167.5 ± 61.0	190.1 ±61.7*	183.8 ±70.0	206.5 ±87.4†	188.6 ±59.7
Plasma Aβ-42					
Aβ-42 in 1998–1999	17.3 ±13.4	24.5 ±16.0†	18.9 ±9.9	21.0 ±16.1	24.9 ±12.3
Aβ-42 in 2002–2003	23.0 ±15.5	29.4 ±19.8*	25.9 ±11.4	23.8 ±16.7	22.2.3 ±8.6
Aβ-42/Aβ-40 ratio					
1998–1999	0.11 ±0.06	0.14 ±0.07*	0.13 ±0.05	0.12 ±0.05	0.14 ±0.05
2002–2003	0.13 ±0.07	0.15 ±0.06	0.13 ±0.06	0.11 ±0.06	0.12 ±0.05

Data are presented as mean ±SD or no. (%).

t test and χ^2:

* $p < 0.05$;

† $p < 0.01$;

≠ $p < 0.001$ (see text).

§ Told by doctor.

¶ By American Diabetic Association.

|| History of myocardial infarction, angina, or congestive heart failure.

MCI = mild cognitive impairment; 3MSE = Modified Mini-Mental State Examination.

Table 2

Relationship between Aβ levels in 1998–1999 and incident dementia in 2002–2003 in normal subjects (all subjects)

	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Aβ-40	1.46 (1.03–2.08)	0.03	1.33 (0.93–1.73)	0.12	1.16 (0.78–1.71)	0.47	1.32 (0.74–1.86)	0.25
Aβ-42	1.61 (1.14–2.28)	0.007	1.47 (1.03–2.10)	0.03	1.35 (0.93–1.97)	0.11	1.46 (0.96–2.22)	0.08
Aβ-42/Aβ-40	1.40 (0.99–1.97)	0.05	1.33 (0.93–1.89)	0.11	1.33 (0.93–1.90)	0.12	1.37 (0.91–2.06)	0.12

Model 1: unadjusted; Model 2: adjusted for age; Model 3: adjusted for age and cystatin C level; Model 4: adjusted for age, Modified Mini-Mental State Examination scores, cystatin C, APOE-4, and MRI identified infarcts. The odds ratios (ORs) were estimated with increments of 1 SD of plasma Aβ1-40 and Aβ1-42.

Table 3

Relationship between Aβ levels in 1998–1999 and incident mild cognitive impairment in 2002–2003 in normal subjects (all subjects)

	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Aβ-40	1.39 (0.99–1.96)	0.05	1.34 (0.95–1.89)	0.09	1.47 (1.00–2.16)	0.05	1.61 (1.03–2.53)	0.03
Aβ-42	1.30 (0.93–1.83)	0.13	1.27 (0.90–1.79)	0.17	1.28 (0.90–1.83)	0.17	1.34 (0.90–1.99)	0.14
Aβ-42/Aβ-40	1.07 (0.78–1.49)	0.66	1.07 (0.77–1.49)	0.66	1.04 (0.75–1.45)	0.80	1.06 (0.74–1.54)	0.74

Model 1: unadjusted; Model 2: adjusted for age; Model 3: adjusted for age and cystatin C level; Model 4: adjusted for age, Modified Mini-Mental State Examination scores, cystatin C, APOE-4, and MRI identified infarcts. The odds ratios (ORs) were estimated with increments of 1 SD of plasma Aβ1-40 and Aβ1-42.

Table 4

Cross-sectional analysis: Relationship between Aβ levels in 2002–2003 and diagnosed dementia in 2002–2003

	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	***p* Value**	**OR (95% CI)**	***p* Value**	**OR (95% CI)**	***p* Value**	**OR (95% CI)**	***p* Value**
Aβ-40	1.45 (1.03–2.04)	0.03	1.35 (0.95–1.92)	0.09	1.29 (0.88–1.90)	0.19	1.33 (0.82–2.16)	0.25
Aβ-42	1.43 (1.04–1.96)	0.02	1.38 (1.00–1.91)	0.05	1.34 (0.95–1.91)	0.10	1.30 (0.87–1.94)	0.21
Aβ-42/Aβ-40	1.29 (0.92–1.80)	0.13	1.28 (0.90–1.23)	0.16	1.26 (0.89–1.79)	0.18	1.19 (0.81–1.75)	0.37

Model 1: unadjusted; Model 2: adjusted for age; Model 3: adjusted for age and cystatin C level; Model 4: adjusted for age, Modified Mini-Mental State Examination scores, cystatin C, APOE-4, and MRI identified infarcts. The odds ratios (ORs) were estimated with increments of 1 SD of plasma Aβ1-40 and Aβ1-42