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Plasma amyloid β and risk of Alzheimer's disease in the Framingham Heart Study

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Abstract

Background—Plasma amyloid β (Aβ) peptides levels have been examined as a low-cost, accessible marker for risk of incident Alzheimer's disease (AD) and dementia, but results have

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Conflict of Interest Disclosures

None reported.

Role of the Sponsor

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varied between studies. We reassessed these associations in one of the largest, prospective, community-based studies to date.

Methods—A total of 2189 dementia-free, Framingham Study participants over age 60 years (mean age 72±8; 56% women) had plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ measured and were followed prospectively (mean 7.6±3.0 years) for dementia/AD.

Results—Increased plasma $A\beta_{1-42}$ levels were associated with lower risk of dementia (Hazard ratios: $A\beta_{1-42}$ HR=0.80 [0.71–0.90], p<0.001; $A\beta_{1-42}/A\beta_{1-40}$ ratio HR=0.86 [0.76–0.98], p=0.027) and AD ($A\beta_{1-42}$ HR=0.79 [0.69–0.90], p<0.001; $A\beta_{1-42}/A\beta_{1-40}$ ratio HR=0.83 [0.72–0.96], p=0.012).

Conclusion—Our results suggest that lower plasma Aβ levels are associated with risk of incident AD and dementia. They encourage further evaluation of plasma Aβ levels as a biomarker for risk of developing clinical AD and dementia.

Keywords

Aβ peptides; plasma biomarker; incident Alzheimer's disease; incident dementia; Framingham heart study; epidemiology; meta-analysis

1 Background

Dementia is a major public health problem with worldwide prevalence expected to reach 115.4 million persons by 2050, representing an enormous societal and financial burden to affected individuals, their families and the health care systems they will need to access [1]. Dementia due to Alzheimer's Disease (AD) represents 50 to 70% of all dementia and thus, successfully targeting this pathophysiology should help diminish the burden of dementia. Therapeutic trials have, so far, had very limited success, likely because brain damage builds up during a long preclinical phase [2] so that treatment starting at the time of the clinical dementia diagnosis comes when those lesions are already irreversible. Historically, visualization ofAD neuropathology was only possible through post-mortem examination of the brain, allowing a definite diagnosis. Recently, several biomarkers have become available and allow assessment of the activity of AD pathophysiological processes in living patients, before the onset of clinical dementia [3].

Among these hallmark pathophysiological processes, abnormal production and aggregation of amyloid β (Aβ) isoforms (mainly $A\beta_{42}$ and $A\beta_{40}$) in the brain is one of the earliest, beginning several decades before onset of clinical symptoms [4] and triggering a cascade of events leading to synaptic loss, neuronal death and clinical dementia [5]. Thus, following the activity of the amyloid process in asymptomatic subjects could be useful to select those persons at higher risk of developing AD, for inclusion in clinical trials of putative treatments for AD and in the future, for preventive interventions [6].

Currently, Aβ peptide concentrations are most frequently detected in body fluids, by measuring Aβ levels in the cerebrospinal fluid (CSF), or through brain imaging of amyloid deposition. CSF levels fall in parallel with increased brain deposition as AD pathology accumulates and thus, can help refine the differential diagnosis in subjects with clinical

dementia [7,8] and identify healthy elderly and individuals with mild cognitive impairment (MCI) who are at high risk of developing AD in the future [9–11]. But lumbar puncture is invasive and amyloid imaging expensive, restricting their wide-spread or frequent use in large populations of asymptomatic subjects.

Given their greater accessibility, there is considerable interest in examining whether circulating Aβ levels correlate with AD risk. A recent meta-analysis by Koyama *et al* and subsequent studies suggest such an association [12–15] but high inter-assay variability, differences in study design and follow-up time have led to significant heterogeneity and conflicting results [14, 16].

In order to help clarify this matter, we measured plasma $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ in a prospective, community-based, cohort under ongoing surveillance for AD, using a validated, commercially available amyloid assay. Our main objective was to assess longitudinal associations between plasma Aβ peptides levels and risk of incident dementia and AD in our study. Our secondary objective was to update the meta-analysis published by Koyama et al [14].

2 Methods

2.1. Study Sample

The Framingham Heart Study (FHS) is an ongoing community-based prospective cohort study of cardiovascular disease and its risk factors. It was initiated in 1948 with the enrollment of 5209 women and men aged 28 to 74 years (Original Cohort) [17]. Original cohort participants are reassessed biennially at a comprehensive core examination and have been examined 31 times to date [18]. In 1971, offspring of the original cohort and the spouses of these offspring (n = 5124, age 5–70 years, 3548 biological offspring, 1576 offspring spouses) were enrolled in the Framingham Offspring Cohort [19]. They have been examined every 4 to 8 years since, 9 times to date, for a core examination [20]. In addition, both cohorts have been under ongoing surveillance for cognitive decline and dementia since 1975.

A total of 4,039 participants who attended the 23rd Original cohort examination (1992–1996, n=772) or the 7th Offspring examination (1998–2001, n=3267) had plasma $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ measured. We excluded participants aged < 60 years (n=1532) as they were unlikely to develop late-onset Alzheimer's disease, and to be more consistent with other studies. We also excluded participants with prevalent dementia (n=42) or with no follow-up (n=276), yielding a subsample of 2189 participants for longitudinal assessment of dementia and Alzheimer's disease risks related to plasma Aβ concentrations.

The study protocol was approved by the Institutional Review Board of the Boston University Medical Center and all participants provided written informed consent.

2.2. Plasma Aβ Assessment

EDTA plasma specimens used for the Aβ analyses were drawn into K3-EDTA evacuated specimen tubes, in the early afternoon in a supine nonfasting state for Original cohort, and in

the morning in a supine fasting state for Offspring cohort. Specimen tubes were centrifuged for 30 minutes at 1850g at 4 degrees Celsius. Plasma was then separated from cells after centrifugation and placed at −80 degrees Celsius, within 90 minutes of venipuncture. The original aliquots consisted of 2mL of plasma in 3mL cryogenic storage vials for Original cohort samples and 700 μL of plasma in 1mL cryogenic storage vials for Offspring cohort samples. All specimens were stored at −80 degrees Celsius until they were aliquoted in March 2012 to be frozen and shipped for the assay. Therefore, specimens were thawed once prior to Aβ measurement. New aliquots consisted of 150 μL of plasma in 0.5 mL cryogenic storage vials. All samples were analyzed at the Department of molecular pharmacology and experimental therapeutics of the Mayo Clinic, Jacksonville, FL, from June to August 2012. Quantification of Aβ isoforms in plasma was performed using INNO-BIA plasma Aβ forms assays (Innogenetics, Ghent, Belgium), which is a multiplex microsphere-based Luminex xMAP technique that allows simultaneous analysis of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ [21]. Measurements were done in duplicate in a randomly selected sample representing 9% of all samples. Intra-assay coefficients of variations (CV) for $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were 3.2% and 2.6% and inter-assay CVs were 10.5% and 7.6%, respectively. Analysis of 146 phantom samples showed intraclass correlation coefficients of 0.916 and 0.943 and CV of 4.8% and 3.5%, respectively.

2.3. Dementia and Alzheimer's Disease Diagnosis

We screened participants at each examination for possible cognitive decline through a number of mechanisms, including an administration of the Folstein Mini-Mental Status Examination (MMSE) [22], Briefly, a MMSE score below the education-specific cutoff score (which ranged from ≤26 in college-educated persons to ≤23 in persons with less than a high school education), a decline of 3 or more points between two successive administrations, or a decline of more than 5 points compared with any previous examination prompted further in-depth testing. Other mechanisms included referral by FHS staff and physicians at regular clinic exams, by self, family or primary care physician, referral following health updates or ancillary studies by other FHS working groups, and referral based on performance on two neuropsychological test batteries administered 6 years apart to most participants as part of an independent initiative [20, 23]. Participants with a stroke diagnosis were also examined at 6, 12 and 24 months for possible cognitive decline. Persons "flagged" as having possible MCI or otherwise being at risk for developing dementia underwent a more detailed neuropsychological evaluation, including the Logical Memory test from the Wechsler Memory Scale, the Similarities test from the Wechsler Adult Intelligence Scale, the Trail-Making Tests A and B, Boston Naming Test, Controlled Word Association Test, Hooper Visual Organization Test, Clock Drawing Test, and Wide Range Achievement Test. A neurological examination was also performed, blinded from the neuropsychological evaluation results. If the neuropsychological testing or neurological evaluation suggested a decline in cognitive function, and other sources of data could not clarify if the person had MCI or AD, we administered a structured family interview and all persons were assigned a Clinical Dementia Rating [24] scale score. We then determined whether each person fulfilled criteria for a diagnosis of dementia, the probable date of onset and type of dementia at a consensus review conducted by a panel comprising at least one behavioral neurologist and one neuropsychologist. The panel reviewed all available records

including examinations by FHS investigators, hospital and nursing home records, data from structured family interviews, imaging and when available, autopsy data. Participants with dementia met criteria outlined in the Fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria [25], and were required to have symptoms for at least 6 months. Participants with AD met National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association criteria for definite, probable or possible AD [26]. For the present analyses, data for incident dementia obtained till December 2012 were used.

2.4. Statistical Analysis

We log-transformed plasma Aβ levels ($A\beta_{1-42}$, $A\beta_{1-40}$) in order to account for the skewness of their distributions. Plasma Aβ ratio was computed as [$A\beta_{1-42}/A\beta_{1-40}$], and then log-transformed. Plasma Aβ levels were either studied as a continuous variable and in that case were also standardized to reflect risk associated with a difference of 1 standard deviation (SD) in baseline levels, or as quartiles.

Longitudinal associations of plasma Aβ levels with risk of incident dementia and AD were assessed using Cox regression models. In the dementia analysis, participants were followed from the baseline examination until they developed dementia, died or had been followed for 10-years. Persons whose cognitive status at 10 years after baseline was not known were censored at the time they were last documented to be free of dementia (n=960). In the AD analysis, additional right censoring at time of other dementia diagnosis was performed. Successive adjustment strategies were used, namely: adjustment for age and gender (Model A); further adjustment for education, APOE ε4 status (model B); and for prevalent hypertension, diabetes and cardiovascular disease, current smoking, total cholesterol levels and being in the top sex-specific quartile of waist-hip ratio (model C). In threshold models, the survival without AD was also estimated from model A within each quartile of plasma Aβ levels. We also performed adjustment for creatinine levels in a sensitivity analysis, as data were not available in all participants (n=2018). We computed Spearman correlation coefficients of creatinine levels with plasma Aβ concentrations, and a Cox regression model adjusted for age, gender and creatinine levels (model A+). We could not adjust for platelet counts as these had not been measured at the same examinations as plasma Aβ levels.

We then examined whether plasma Aβ concentrations improved AD risk prediction when added to models with age, gender, education and APOEε4 by computing continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) [27, 28]. Those measures and their confidence intervals were estimated using bootstrap to account for the risk of overfitting, and interpreted by comparing their values to simulated data [29], Briefly, we used IDI≈0.004 and NRI≈0.16, IDI≈0.024 and NRI≈0.40 and IDI≈0.06 and NRI≈0.62 to characterize small, moderate, and large improvements, respectively. Finally, we updated the results of the meta-analysis published by Koyama *et al* [14]. Using the same selection of prospective cohorts, we first extracted hazard ratios of incident AD or dementia and their 95% confidence intervals from minimally adjusted models comparing lowest versus highest quantiles (quartiles or tertiles) of plasma $A\beta_{1-42}$ levels, plasma $A\beta_{1-40}$ levels or plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio. In addition to our results and

those published by Shah *et al* [15], and Lui *et al* [12], we contacted three previously published studies, including Shah *et al*, that had measured plasma Aβ levels, to obtain unpublished hazard ratios [13, 15, 30]. Lastly, we performed fixed- and random-effect meta-analyses, and evaluated heterogeneity using the I^2 measure.

Cox regression analyses were performed using Statistical Analysis System software version 9.2 (SAS Institute, Cary, NC). Meta-analyses were performed using R version 3.02 and the metafor package [32]; the latter were also used to create survival curves and forest plots.

3 Results

After up to 10 years of follow-up (mean time ± standard deviation: 7.6 ± 3.0 years; median time: 9 years), 237 participants were diagnosed with dementia (194 had AD). The main characteristics of the study population are summarized in table 1.

Longitudinal associations between plasma Aβ levels and risk of incident AD and dementia are presented in table 2. Increased plasma $A\beta_{1\text{-}42}$ levels were associated with lower risks of incident AD and dementia after adjustment for age and gender. An increase of one standard deviation of plasma $A\beta_{1\text{-}42}$ levels was associated with a decrease of 21% in incident AD risk (hazard ratio (HR)=0.79; 95% confidence interval (CI) [0.69–0.90]; $p=3.00 \times 10^{-04}$) and 20% in incident dementia risk (HR=0.80; 95% CI [0.71–0.90]; $p=2.00 \times 10^{-04}$). Risks of AD by quartiles of plasma $A\beta_{1\text{-}42}$ levels are presented in table 2 and figure 1. Compared to participants from the fourth quartile (highest levels), participants from the first quartile of plasma $A\beta_{1\text{-}42}$ had a greater than two-fold risk of AD (HR=2.46; 95% CI [1.60–3.78]) and dementia (HR=2.20; 95% CI [1.51–3.20]). Similar, although weaker, associations were observed for plasma $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio. No statistically significant association was observed for plasma $A\beta_{1\text{-}40}$ levels.

Associations between plasma $A\beta_{1\text{-}42}$ levels and incident AD and dementia remained significant after additional adjustment for APOE ε4 status, education, hypertension, diabetes, cardiovascular disease, current smoking, total cholesterol levels and waist-hip ratio, and hazard ratios remained stable (see table 2). Similarly, associations between plasma $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio and incident AD remained significant and hazard ratios remained stable. Conversely, associations between plasma $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio and incident dementia became non-significant in Model C. Plasma Aβ levels presented low correlation with creatinine levels, with Spearman coefficients of 0.09, 0.19 and −0.07 for Aβ1-42, Aβ1-40 and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio, respectively. Adjustment for creatinine levels in addition to age and gender did not modify the estimation of hazard ratios (see supplementary Table).

Improvement of AD risk prediction related to plasma Aβ concentrations is illustrated in table 3. Addition of plasma $A\beta_{1\text{-}42}$ concentrations to a model including age, gender, education and APOEε4 status significantly improved AD risk prediction, but the magnitude of this improvement was small to moderate, with a continuous IDI of 0.0105 (95% CI=[0.0036–0.0184]), and a continuous NRI of 0.2592 (95% CI=[0.1152–0.3980]). Updated meta-analyses of reported associations of plasma $A\beta_{1\text{-}42}$ levels, plasma $A\beta_{1\text{-}40}$ levels and plasma $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio and incident AD and dementia are shown in figure 2. Despite high heterogeneity among the cohorts, combining our data with published results from Lui

et al and Shah *et al*, and unpublished results from Shah *et al*, Hansson *et al*, and Lambert *et al*, confirmed the associations between plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio and incident AD and dementia, with random-effect pooled HRs of 1.49 (95% CI=[1.17–1.91]; $p=1.39 \times 10^{-03}$; $I^2=36.71\%$) and 1.53 (95% CI=[1.09–2.15]; $p=1.38 \times 10^{-02}$; $I^2=70.74$), respectively. The association of plasma $A\beta_{1-42}$ levels and incident AD remained non-significant (pooled HR=1.28; 95% CI=[0.82–1.99]; $p=2.79 \times 10^{-01}$; I2=80.86%), and became significant with incident dementia (pooled HR=1.42; 95% CI=[1.03–1.96]; $p=3.35 \times 10^{-02}$; $I^2=68.42\%$), when compared with the previous meta-analysis. The associations of plasma $A\beta_{1-40}$ levels and incident AD and incident dementia were non-significant (pooled HR=1.31; 95% CI=[0.88–1.93]; $p=1.79 \times 10^{-01}$; $I^2=74.13\%$ and pooled HR=1.19; 95% CI=[0.78–1.81]; $p=4.27 \times 10^{-01}$; $I^2=82.33\%$, respectively).

4 Discussion

This study represents one of the largest prospective studies of plasma Aβ levels and risk of incident AD and dementia to date. We found a significant association between low plasma $A\beta_{1-42}$ levels and $A\beta_{1-42}/A\beta_{1-40}$ ratio and higher risk of incident AD and dementia. Combining our results with the existing literature confirmed the associations of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio with incident AD and dementia, despite high heterogeneity.

Low plasma $A\beta_{1-42}$ levels were associated with higher risk of incident AD and dementia in the Framingham cohorts. Conversely, we found no significant association using plasma $A\beta_{1-40}$ levels. Association of plasma Aβ levels with risk of AD and dementia has been debated, due to contradictory results ranging from protective to deleterious, including absence of associations [12–16]. Our results add a new piece of evidence in favor of this association, allowing better estimation of the direction of this association. $A\beta_{42}$ peptides are suspected to be more prone to aggregate and to exert deleterious effects on neurons, than $A\beta_{40}$ peptides [33]. The significant association we found between plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio and incident AD and dementia, although weaker than with plasma $A\beta_{1-42}$, supports the hypothesis that AD results from an imbalance between $A\beta_{1-42}$ and $A\beta_{1-40}$ peptides. The direction of the observed associations is compatible with the hypothesis that late-onset AD results from impaired Aβ clearance from the brain, as opposed to autosomal dominant or Down syndrome forms of AD, in which a relative overproduction of $A\beta_{1-42}$ peptides seems to be involved [34]. Furthermore, the observed associations were noted over a long period of follow-up and consistent with the theory that alterations in Aβ peptides are an early event in the pathophysiology of AD [2]. Part of the Aβ pool in plasma could come from platelets [35], and whether plasma Aβ can reflect the aggregation of Aβ in the brain is still debated. In favor of the latter are recent reports of associations of low plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio with increased amyloid brain uptake, as measured by Pittsburgh compound B (PiB) PET scan [36–38]. Brain PiB PET was not available in FHS at the time of this study and we could not adjust for platelet counts as they had been measured at different exams. Moreover, given that the vast majority of dementia cases were Alzheimer's disease in FHS, it is difficult to assess the specificity of plasma Aβ levels to AD. In the meta-analysis, effect sizes tended to be higher with dementia than AD, but as the set of studies used differed and the confidence intervals greatly overlapped, further studies are needed to answer this question, especially

since Aβ levels are much more likely to reflect the mechanistic pathway for AD than other dementias.

Updated meta-analysis confirmed the associations of plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio with incident AD and dementia, and association of plasma $A\beta_{1-42}$ levels with incident dementia became significant. Despite these improved results, high heterogeneity was important in all meta-analyses. Differences in blood sampling (fasting versus non fasting, time of the day, tube used for collecting blood) and storage protocols may account for a substantial portion of the observed heterogeneity [16]. Moreover, assays may differ in their capacity to capture Aβ peptides, and most of them can only detect free, circulating monomers, which represent less than 50% of all circulating Aβ peptides [39]. Finally, sample size, follow-up time and analysis strategies may also generate differences in association estimates. Furthermore, other studies of interest could not be included in this meta-analysis as they did not report risk of AD or dementia according to quantiles of plasma Aβ levels [40–42], In 274 non-demented participants from the Cardiovascular Health Study, Lopez *et al* reported high plasma Aβ levels and ratio at baseline in those with incident dementia after 4.5 years of follow-up, but those results became not significant after multivariable adjustment [41]. In 585 participants from the Vienna Transdanube Aging study, Blasko *et al* reported higher levels of plasma $A\beta_{42}$ in cognitively normal participants who converted to MCI or AD after 2.5 years of follow-up compared to participants who remained cognitively stable [40]. In the Alzheimer's Disease Neuroimaging Initiative, no significant association between plasma Aβ levels or ratio and convertion to MCI/AD was reported [42], Therefore, there is a need for standardized protocols for blood sampling and storage, improved assays and collaborative efforts of analysis, re-analysis and meta-analysis to tackle the heterogeneity problems that have hampered the study of plasma Aβ peptides so far.

Study of net reclassification improvement and integrated discrimination improvement showed a modest improvement in prediction of AD risk over the integrative predictive value of age, gender, education, and APOEε4. These results suggest that plasma Aβ concentrations could be used as a biomarker for risk prediction of AD and dementia, more likely as part of a panel of relevant biomarkers. A study has recently observed that using plasma Aβ concentrations in combination with other plasma biomarkers improved prediction of neocortical Aβ burden over age, APOEε genotype and Clinical Dementia Rate Scale sum of boxes [43].

Our study has several strengths: with more than 2000 participants, it is one of the largest studies of plasma Aβ levels to date. The Framingham Heart Study is a prospective cohort, with a long period of follow-up, and, as a single center study, had homogeneous and standardized procedures for dementia assessment. We used a commercially available assay of plasma Aβ levels, which, we hope, will facilitate reproducibility of our results and meta-analysis. Finally, we tried to account for possible confounding factors in our statistical analysis.

The main limitations include the differences in blood sampling conditions between the Original and the Offspring cohorts, which could confound the real association between plasma Aβ levels and risk of AD and dementia, the largely European sample, which limits

generalizability to other ethnicities, and the lack of repeated measures of plasma Aβ concentrations. Aβ aggregation is a slow and dynamic process and repeated measures may better estimate the risk of incident AD as well as the best timing for preventive intervention [44].

Overall, these results suggest that lower plasma Aβ42 and Aβ40 levels precede and are associated with risk of incident AD and dementia. They encourage further evaluation of plasma amyloid β levels as a potential biomarker for preclinical AD and risk of developing clinical AD and dementia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Three City Study

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Prospective Population Study of Women and Gerontological (PPSW) and Geriatric Population (H70) Studies

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Research in context

1. Systematic review

A low-cost, accessible, and efficient biomarker for incipient Alzheimer's disease is needed. We found that low plasma $A\beta_{1-42}$ levels are associated with high risk of incident Alzheimer's disease and dementia in 2189 participants over age 60 that were followed prospectively for up to 10 years in the Framingham Heart Study. We also meta-analyzed our results with data from prior publications.

Interpretation

This work confirms the suspected associations between plasma $A\beta_{1-42}$ levels and risk of incident AD and dementia. Plasma Aβ levels could represent a useful biomarker for preclinical Alzheimer's disease and dementia.

3. Future directions

Further studies are needed to confirm these associations, and to evaluate the added value of plasma Aβ levels in terms of risk prediction, whether alone, or as part of a biomarker panel. This will require further standardization of procedures for samples collection and conservation, plasma Aβ assays, study design and analyses.

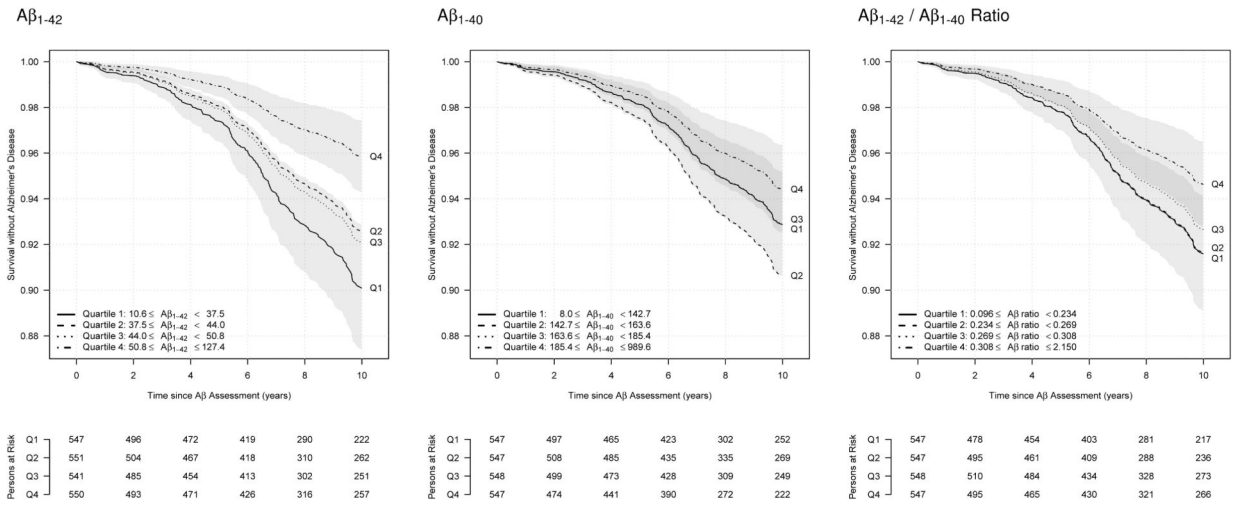

Figure 1.

Survival without Alzheimer's disease according to quartiles of plasma $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio

NOTES. Survival is derived from a Cox model adjusted for age and gender. 95% confidence intervals are represented for first and fourth quartiles.

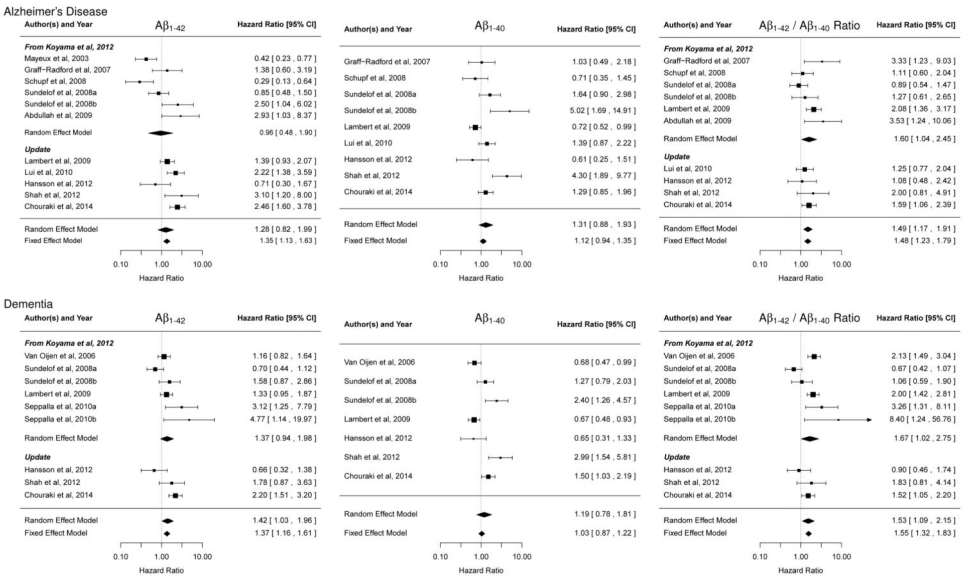

Figure 2.

Meta-analysis of plasma $A\beta_{1\text{-}42}$ levels, plasma $A\beta_{1\text{-}40}$ levels and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio and risk of incident Alzheimer's disease and dementia (updated from Koyama *et al*, 2012)

Table 1

Characteristics of the study population at baseline

	Population at risk for dementia (n=2189)
General characteristics	
Age, years, mean (standard deviation)	72.16 (7.57)
Women, % (N)	56.28 (1232)
Education, % (N)	
No high school degree	12.84 (276)
High school degree	34.74 (747)
Some college	26.65 (573)
College graduate	25.77 (554)
APOE ε4 genotype : ≥ 1 APOE ε4 allele, %	21.51 (465)
Confounders	
Hypertension, % (N)	61.17 (1359)
Cardiovascular disease, % (N)	24.17 (529)
Smoking, % (N)	7.59 (166)
Total cholesterol, mg/dL, mean (standard deviation)	201.19 (36.63)
Diabetes, % (N)	15.12 (327)
Plasma Aβ levels	
$A\beta_{1-42}$ levels (pg/mL)*	44.0 (37.5–50.8)
$A\beta_{1-40}$ levels (pg/mL)*	163.6 (142.7–185.4)
$A\beta_{1-42}/A\beta_{1-40}$ ratio*	0.268 (0.234–0.308)

mean (standard deviation) are presented unless otherwise stated

*Those variables were log-transformed in the analyses. Thus, the median (interquartile range) is used instead of mean (standard deviation)

Table 2

Association of plasma Aβ levels with risk of incident dementia and Alzheimer's disease

		Continuous (per SD)			Quartile 1		Quartile 2		Quartile 3		Quartile 4
		HR	**95% CI**	**P**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**HR**
Model A*											
$A\beta_{1-40}$	AD	0.93	[0.81–1.06]	2.59×10^{-01}	1.29	[0.85–1.96]	1.71	[1.17–2.51]	1.28	[0.84–1.96]	Ref.
	Dementia	0.91	[0.81–1.03]	1.26×10^{-01}	1.50	[1.03–2.19]	1.58	[1.10–2.27]	1.48	[1.01–2.16]	Ref.
$A\beta_{1-42}$	AD	0.79	[0.69–0.90]	3.00×10^{-04}	2.46	[1.60–3.78]	1.82	[1.18–2.81]	1.94	[1.26–3.00]	Ref.
	Dementia	0.80	[0.71–0.90]	2.00×10^{-04}	2.20	[1.51–3.20]	1.52	[1.03–2.24]	1.66	[1.13–2.44]	Ref.
$A\beta_{1-42}/A\beta_{1-40}$ ratio	AD	0.83	[0.72–0.96]	1.23×10^{-02}	1.59	[1.06–2.39]	1.58	[1.05–2.38]	1.38	[0.90–2.12]	Ref.
	Dementia	0.86	[0.76–0.98]	2.73×10^{-02}	1.52	[1.05–2.20]	1.48	[1.02–2.14]	1.39	[0.95–2.04]	Ref.
Model B†											
$A\beta_{1-40}$	AD	0.93	[0.81–1.05]	2.51×10^{-01}	1.30	[0.85–1.99]	1.78	[1.21–2.62]	1.33	[0.86–2.04]	Ref.
	Dementia	0.92	[0.82–1.03]	1.65×10^{-01}	1.45	[0.99–2.12]	1.64	[1.14–2.36]	1.53	[1.04–2.24]	Ref.
$A\beta_{1-42}$	AD	0.79	[0.69–0.90]	4.00×10^{-04}	2.36	[1.53–3.64]	1.81	[1.17–2.81]	1.86	[1.20–2.87]	Ref.
	Dementia	0.81	[0.72–0.91]	6.00×10^{-04}	2.06	[1.41–3.01]	1.53	[1.04–2.26]	1.63	[1.11–2.40]	Ref.
$A\beta_{1-42}/A\beta_{1-40}$ ratio	AD	0.84	[0.72–0.97]	1.55×10^{-02}	1.58	[1.05–2.38]	1.47	[0.98–2.22]	1.29	[0.84–1.99]	Ref.
	Dementia	0.87	[0.77–1.00]	4.28×10^{-02}	1.47	[1.01–2.12]	1.38	[0.95–2.00]	1.32	[0.90–1.93]	Ref.
Model C‡											
$A\beta_{1-40}$	AD	0.92	[0.81–1.05]	2.28×10^{-01}	1.30	[0.84–2.01]	1.88	[1.27–2.78]	1.29	[0.83–2.00]	Ref.
	Dementia	0.92	[0.81–1.03]	1.44×10^{-01}	1.44	[0.97–2.13]	1.73	[1.20–2.51]	1.49	[1.01–2.20]	Ref.
$A\beta_{1-42}$	AD	0.79	[0.68–0.90]	5.00×10^{-04}	2.31	[1.49–3.58]	1.67	[1.06–2.63]	1.75	[1.12–2.75]	Ref.
	Dementia	0.81	[0.72–0.92]	1.20×10^{-03}	1.95	[1.32–2.87]	1.40	[0.94–2.09]	1.53	[1.03–2.27]	Ref.
$A\beta_{1-42}/A\beta_{1-40}$ ratio	AD	0.85	[0.73–0.98]	2.72×10^{-02}	1.59	[1.05–2.40]	1.38	[0.91–2.09]	1.27	[0.81–1.99]	Ref.
	Dementia	0.89	[0.78–1.02]	8.57×10^{-02}	1.44	[0.99–2.10]	1.27	[0.87–1.85]	1.29	[0.86–1.92]	Ref.

Abbreviation: AD, Alzheimer's disease; HR, Hazard ratio; SD, Standard deviation; 95% CI, 95% Confidence interval; Ref., Reference

NOTE. Hazard ratios, confidence intervals and P-value were evaluated in Cox regression models

* Model A: adjusted for age and gender

† Model B: adjusted for age, gender, education and APOE ε4 status

‡ Model C: adjusted for age, gender, education, APOE ε4 status, prevalent hypertension, diabetes and cardiovascular disease, current smoking, total cholesterol levels, and being in the top sex-specific quartile of waist-hip ratio

Table 3

Alzheimer's disease risk prediction improvement of plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels

	Aβ1-40 – Continuous (SD)	Aβ1-40 – Q4 vs Q123	Aβ1-42 – Continuous (SD)	Aβ1-42 – Q4 vs Q123
Continuous				
IDI [95% CI]	0.0015 [−0.0011–0.0043]	0.0062 [−0.0005–0.0130]	0.0105 [0.0036–0.0184]	0.0046 [−0.0032–0.0127]
NRI [95% CI]	0.1632 [0.0154–0.2976]	0.0338 [−0.0929–0.1594]	0.2592 [0.1152–0.3980]	0.1298 [0.0198–0.2437]

Abbreviations: SD, Standard deviation; Q4, 4th quartile; Q123, combined 1st, 2nd and 3rd quartiles, IDI, Integrated discrimination improvement; NRI, Net reclassification improvement; 95% CI, 95% Confidence interval

NOTE. Improvement was evaluated when adding the measure of interest in a Cox regression model of risk of incident Alzheimer's disease adjusted for age, gender, education and APOEε4