Meta-Analysis of Plasma Amyloid-β levels in Alzheimer's Disease

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Abstract

Plasma amyloid-β (Aβ) levels have been proposed as biomarkers of Alzheimer's disease (AD), but studies have produced inconsistent results. We present a meta-analytic review of cross-sectional studies that examined plasma Aβ levels in AD and cognitively normal subjects, and longitudinal studies that used baseline plasma Aβ levels to predict conversion from normal cognition to AD. Medline and EMBASE databases were searched to generate an initial list of relevant studies, and selected authors approached for additional data. Twelve cross-sectional studies ($n = 1483$) and seven longitudinal ($n = 3920$) met the inclusion criteria for meta-analysis. Random effects model was used to calculate the weighted mean difference (WMD) by Review Manager Version 4.2. In longitudinal studies, cognitively normal individuals who converted to AD had higher baseline $A\beta_{1-40}$ and $A\beta_{1-42}$ levels (WMD: 10.29, $z = 3.80$, $p = 0.0001$ and WMD: 8.01, $z = 2.76$, $p = 0.006$, respectively), and non-significantly increased $A\beta_{1-42}/A\beta_{1-40}$ ratio (WMD: 0.03, $z = 1.65$, $p = 0.10$). In cross sectional studies, compared to cognitively normal individuals, AD patients had marginally but non-significantly lower $A\beta_{1-42}$ levels (WMD:−2.84, $z = 1.73$, $p = 0.08$), but $A\beta_{1-40}$ levels were not significantly different (WMD: 3.43, $z = 0.40$, $p = 0.69$). Our systematic review suggests a model of differential longitudinal changes in plasma Aβ levels in cognitively stable individuals versus those who go on to develop AD dementia. Baseline $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in cognitively normal elderly individuals might be predictors of higher rates of progression to AD, and should be further explored as potential biomarkers.

Keywords

Alzheimer's disease; amyloid-β; meta-analysis; plasma

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INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and is generally diagnosed in old age. Pathophysiological changes of AD, however, start many years and possibly even decades before cognitive impairment becomes clinically apparent [1, 2]. The amyloid cascade hypothesis is currently the dominant explanation of AD etiology. This hypothesis suggests that a chronic imbalance between production and clearance of the amyloid-β (Aβ) peptide results in plaque deposition [3]. Markers of abnormal Aβ processing may therefore represent early, and even preclinical, markers of AD, especially the more pathogenic 42-amino-acid isoform $A\beta_{1-42}$.

There is growing evidence that low $A\beta_{1-42}$ in cere-brospinal fluid (CSF) could be a biomarker for the diagnosis of AD. Several studies have consistently shown that the concentration of $A\beta_{1-42}$ in CSF is low in AD patients compared with healthy controls [4–7]. A meta-analysis of CSF studies showed that levels of $A\beta_{1-42}$ were significantly lower in AD [6] and this change was also related to the conversion from mild cognitive impairment (MCI) to AD [8, 9].

Plasma sampling is simpler and less invasive than a lumbar puncture, and particularly well suited to use in aging samples or when multiple measures are required, such as in clinical trials. However, the published data on plasma Aβ levels in AD is conflicting. In cross-sectional studies, plasma Aβ levels of AD have been reported to be higher, lower, or unchanged, and there are few large-scale longitudinal studies that relate plasma Aβ to the prospective risk of dementia. To our knowledge, no meta-analysis of plasma Aβ levels in AD has been completed. We therefore performed a meta-analysis to examine: 1) whether plasma Aβ levels were significantly different between AD and cognitively normal subjects in cross-sectional studies, and 2) whether baseline plasma Aβ levels were associated with conversion from cognitively normal to incident AD in longitudinal studies.

METHODS

Data sources

A systematic literature search of MEDLINE, EMBASE, the Cochrane Library, and Psychoinfo was conducted for studies published in the period from January 1989 to July 2010. Our search strategy included the Medical Subject Headings (MeSH) and text keywords: amyloid beta protein or amyloid beta, plasma and Alzheimer's disease. It generated a total of 119 articles. Abstracts were reviewed, and a large number of these were excluded on the grounds of covering an irrelevant topic or lacking original data. The remaining thirty four studies underwent further assessment for suitability. Two reviewers (F.S. and A.P.) independently collected data, and data disagreement was resolved through discussion. Where additional information was required, the corresponding authors were contacted for clarification or additional data. Of the seven studies for which the corresponding author was contacted, authors of five studies replied with relevant data and information [10–14].

Study selection

We chose to focus on AD and excluded studies of any other type of dementia. For longitudinal studies, we chose those studies with cognitively normal subjects at the baseline with progression to AD. A study was included if it met the following criteria:

1. Provided detailed procedure and criteria for the diagnosis of AD.

2. Total number of participants >30.

3. Reported Mean, Standard Deviation (SD) or Standard Error (SE) on plasma $A\beta_{1-40}$, $A\beta_{1-42}$ levels.

4. If cross-sectional, the study provided both control and AD $A\beta$ levels.

5. If longitudinal, the study provided information about conversion to AD from a baseline of cognitively normal status.

6. Included both males and females.

7. Did not include studies of familial AD.

Twelve cross sectional and seven longitudinal studies met the inclusion criteria and were used in this meta-analysis. If a study fulfilled all inclusion criteria but did not report all relevant data, we contacted the authors to obtain the additional data. (For data details, see Tables 1, 2).

Statistical analysis

Statistical analysis was performed using weighted mean difference (WMD) methodology in Review Manager (Version 4.2 for Windows. Copenhagen, Denmark: The Nordic Cochrane Centre, the Cochrane Collaboration, 2003). The Random effects model was used to calculate the overall effect. WMD is the pooled difference between disease and control groups on mean values across a group of studies using the same scale of measurement for the outcome. For cross-sectional studies, the meta-analysis compared WMD between the AD subject group and the cognitively normal group. For longitudinal studies, WMD of baseline $A\beta$ levels was calculated between individuals who converted to AD and those who remained normal. We also analyzed data using standardized mean difference (SMD) which the difference between two estimated means divided by an estimate of the standard deviation, and compared the results of WMD and SMD. Publication bias was investigated using funnel plots, with a roughly symmetrical funnel plot suggesting a lack of bias. If a plot does not resemble an inverted funnel, it usually suggests a publication bias. As discussed below however, other factors may result in an asymmetrical plot.

RESULTS

Included studies

Twelve cross sectional studies presented $A\beta_{1-42}$ levels for AD and cognitively normal subjects, and eleven provided $A\beta_{1-40}$ levels [11, 13, 15–24]. Only two studies provided the $A\beta_{1-42}/A\beta_{1-40}$ ratio [11, 17], and two presented the $A\beta_{1-40}/A\beta_{1-42}$ ratio [18, 20]. Seven longitudinal studies provided data on baseline $A\beta_{1-42}$ levels on subjects with incident AD

who converted from cognitively normal; and six of the seven studies also presented $A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratios [10,12, 14, 25–28]. Further details of included studies are provided in Tables 1, 2.

Aβ levels of AD and cognitively normal control subjects in cross-sectional studies

Overall, there was no significant difference in $A\beta_{1-40}$ levels between AD and cognitively normal control subjects (WMD: 3.43, 95%CI: [−13.33, 20.19], $z = 0.40$, $p = 0.69$; SMD: 0.12, 95%CI: [−0.14, 0.39], $z = 0.91$, $p = 0.36$) (Fig. 1A). Compared to controls, AD subjects had a marginally lower $A\beta_{1-42}$ level (WMD: −2.84, 95%CI: [−6.06, 0.38], $z = 1.73$, $p = 0.08$; SMD: −0.12, 95%CI: [−0.32, 0.09], $z = 1.14$, $p = 0.26$) (Figure 1B). Only two studies provided data on the $A\beta_{1-42}/A\beta_{1-40}$ ratio, therefore a meta-analysis was not conducted on this measure.

Baseline Aβ levels in longitudinal studies

Compared with subjects who remained cognitively normal upon follow up, normal-to-AD converters had significantly higher baseline $A\beta_{1-40}$ levels (WMD: 10.29, 95%CI: [4.98, 15.61], $z = 3.80$, $p = 0.0001$; SMD: 0.2, 95%CI: [0.09, 0.31], $z = 3.57$, $p = 0.0004$) (Fig. 2A) as well as baseline $A\beta_{1-42}$ levels (WMD: 8.01, 95%CI: [2.32, 13.70], $z = 2.76$, $p = 0.006$; SMD: 0.33, 95%CI: [0.14, 0.52], $z = 3.43$, $p = 0.0006$) (Fig. 2B), but $A\beta_{1-42}/A\beta_{1-40}$ ratios showed no significant difference (WMD: 0.03, 95%CI: [−0.01, 0.07], $z = 1.65$, $p = 0.10$; SMD: 0.18, 95%CI: [−0.03, 0.40], $z = 1.67$, $p = 0.09$) (Fig. 2C). The differences of baseline $A\beta_{1-40}$ and $A\beta_{1-42}$ levels between normal-to-AD converters and non-converters were moderate ($0.15 < SMD < 0.5$).

Funnel plots of cross-sectional studies

Most of the studies were symmetrical on the funnel plots, although some less precise studies with a large standard error may have caused a publication bias (Fig. 3A, 3B). Two studies were deselected on this basis, Arvanitakis et al. [22] for $A\beta_{1-40}$ comparison and Tamaoka et al. [24] for $A\beta_{1-42}$ comparison, however the results were not significantly affected. There was still no significant difference in $A\beta_{1-40}$ levels between AD and cognitive normal control subjects (WMD: 2.34, 95%CI: [−14.09, 18.78], $z = 0.28$, $p = 0.78$). Compared to control subjects, AD subjects still had a marginally lower $A\beta_{1-42}$ level (WMD: − 2.89, 95%CI: [−5.93, 0.14], $z = 1.87$, $p = 0.06$).

Funnel plots of longitudinal studies

Six of seven $A\beta_{1-42}$ longitudinal studies were the same six studies used for $A\beta_{1-40}$ study comparison, however the funnel plots for each differed greatly (Fig. 3C, 3D). The funnel plot of $A\beta_{1-40}$ longitudinal studies was symmetrical, suggesting a lack of publication bias. However, the funnel plot of $A\beta_{1-42}$ studies (i.e., using the same six studies as the $A\beta_{1-40}$ comparison) was not symmetrical. We therefore labeled the annual conversion rates from non-dementia to incident AD for each $A\beta_{1-42}$ study in the comparison, and found that studies with a high annual conversion rate generated a relatively high baseline of $A\beta_{1-42}$ for AD subjects.

DISCUSSION

As hallmark features of AD, Aβ peptides from different bodily fluids have been the subject of many quantitative studies in order to establish their usefulness in diagnosing and predicting AD. Plasma Aβ measurement in AD has resulted in a variety of outcomes. Unlike previous CSF Aβ meta-analysis [8, 9, 29], we focused only on AD and not other types of dementia, and also evaluated basal Aβ levels in cognitively normal individuals who converted to AD in longitudinal follow up. The resulting meta-analysis showed that AD converters had higher initial plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels in comparison with subjects who remained cognitively normal. Meta-analysis of cross-sectional studies indicated that AD patients had a marginally but non-significantly lower $A\beta_{1\text{-}42}$ level ($p = 0.08$), and $A\beta_{1\text{-}40}$ levels were not significantly different from healthy controls.

Our results are consistent with those studies which could not be included in this systematic review due to the strict entry criteria. Pomara and colleagues followed up cognitively healthy elderly individuals and observed that higher initial plasma $A\beta_{1\text{-}42}$ levels were significantly associated with reductions in cognitive scores during follow-up [30]. Ertekin-Taner et al. found that young non-demented first degree relatives of patients with late onset AD also had elevated plasma Aβ levels [31]. Another study showed that people without dementia and those with prevalent dementia have lower basal $A\beta_{1\text{-}42}$ levels than people with incident dementia [32]. The Rotterdam Study reported that high baseline concentrations of plasma $A\beta_{1\text{-}40}$ were associated with an increased risk of dementia [33]. In the same study, increased $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio was shown to reduce the risk of dementia, however, baseline $A\beta_{1\text{-}42}$ levels alone were not associated with the development of dementia [33]. Furthermore, the meta-analysis of longitudinal studies showed that the differences of baseline $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels between AD converters and subjects who remained cognitively normal were moderate. The clinical implication of Aβ levels as diagnostic biomarkers need to be further investigated.

By contrast, the study by Hansson et al. following MCI at baseline reported that plasma Aβ levels did not show a significant association with progression from MCI to AD [34], while yet another study showed that low plasma $A\beta_{1\text{-}40}$ levels predicted incident AD in elderly men over 70 years old [35]. Similarly, work done by Seppala and coworkers used cognitive decline as a follow-up diagnosis, and reported subjects who declined cognitively during follow-up had lower levels of plasma $A\beta_{1\text{-}42}$ at the baseline [36]. However, these studies were not directly comparable with our meta-analysis data on longitudinal studies because of different cognitive levels at baseline or follow-up, as well as having only single gender participants.

In this meta-analysis, the ratio of baseline $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ in incident AD cases was not significantly different when compared with cognitively stable subjects. Three studies which could not be included in the current meta-analysis reported different results [33, 37, 38]. Okereke et al. observed that higher midlife plasma $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratios were significantly associated with greater late-life decline on the global score of cognitive tests (i.e., lower $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratios were associated with future cognitive decline.) [37]. Graff-Radford and collaborators followed 563 cognitively normal subjects and showed subjects with lower

$A\beta_{1-42}/A\beta_{1-40}$ had a greater risk of developing MCI or AD [38]. The Rotterdam study reported similarly results that an increased $A\beta_{1-42}/A\beta_{1-40}$ ratio was associated with a reduced risk of dementia [33]. However, because these studies used different follow-up diagnoses and statistical treatment of data, we could not include them.

In our cross-sectional analysis, we found that AD patients had marginally lower plasma $A\beta_{1-42}$ levels (WMD: $z = 1.73$, $p = 0.08$), however these did not achieve statistical significance. This is consistent with the statistically significant lower $A\beta_{1-42}$ levels found in most CSF studies. Multiple factors may contribute to the weak trend in the plasma $A\beta_{1-42}$ data of cross sectional studies, such as stages of AD, effects of ageing, *APOE* genotype proportion, and variability in $A\beta$ measurement.

Plasma $A\beta$ levels may vary at different stages of AD. Compared with the early stages of AD, $A\beta_{1-42}$ values were found to be lower in AD subjects in moderate to severe stages of the disease [39]. After follow-up for more than 4 years, AD outpatients with low plasma levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ experienced more rapid cognitive decline [40]. It is hard to control or standardize the AD clinical levels from different cross-sectional studies, so directly comparable $A\beta$ levels of AD could not be determined. Prospective studies observing cognitively normal participants at high risk of AD provide a better understanding of the association between $A\beta$ levels and AD.

The effects of aging and apolipoprotein E (APOE) gene (*APOE)* also complicate the interpretation of plasma $A\beta$ levels [41]. Plasma $A\beta$ may increase in an age-dependent manner in non-demented adults. Lopez et al. found that after 4 years' follow-up, $A\beta$ levels gradually increased with age in cognitively normal subjects [26]. Some studies also showed that increase in age correlated with raised $A\beta$ levels in AD/MCI patients and other neurological controls [21]. Differing proportions of the *APOE* β*4* genotype in the cross-sectional studies might have influenced the plasma $A\beta$ levels. APOE4 altered the clearance and transport of $A\beta$ within different brain compartments and favored the formation of cerebral amyloid angiopathy [42]. APOE isoforms differentially regulated $A\beta$ clearance across the blood-brain barrier [43]. In transgenic animal models, over-expression of *APOE* β4 increased the retention of $A\beta_{1-42}$ in plasma over time in comparison with *APOE* β2 and *APOE* β3, suggesting that the peripheral clearance of $A\beta_{1-42}$ is altered by *APOE* genotype [44].

Measurement of $A\beta$ levels is technically much more challenging in plasma than in CSF, since not only are the $A\beta$ levels approximately ten-fold lower than CSF levels, but the total protein content of plasma is about ten-fold higher. The traditional method of plasma $A\beta$ measurement is sandwich ELISA, however lipoprotein and Fc-binding proteins in plasma may influence immunological detection [45]. Soluble $A\beta$ species also interact and bind to a wide range of proteins, such as albumin, β2-macroglobin, haptoglobin, lipoproteins, and other chaperone proteins [46]. These complexes may interfere with ELISA immunoreactions and confound the results [47]. Furthermore, between-laboratory variability in measurement of plasma $A\beta$ may also be exacerbated by the diversity of ELISA antibodies, with varying sensitivities and sometimes poorly defined specificities. Conventional $A\beta$ ELISAs have been applied to measure monomeric $A\beta$ species [48]. Some $A\beta$ oligomer (oAβ) specific ELISA

assays for plasma have also been developed [49]. Since oAβ has been linked to neuronal toxic effects and synaptic failure, oAβ measurement may support a better understanding of the relationship between different Aβ species in AD *in vivo* [48, 49]. Some assays have been validated in more detail to establish greater sensitivity and selectivity for the assay of plasma Aβ peptides [50].

The dynamic equilibrium between the brain, CSF and plasma Aβ levels also needs to be further clarified. In an animal model, radio labeled $A\beta_{1-40}$ was shown to be rapidly cleared from ventricular CSF to the blood [51], and consistently decreased Aβ levels in CSF and plasma were related to Aβ plaque burden in a mouse model of AD [52]. However, the equilibrium between CSF and plasma Aβ has not been proven in AD patients [16, 53, 54] and cognitive normal individuals [54], and may not be easily verified in humans *in vivo*. Changes to plasma Aβ levels may also be influenced by metabolic turnover time in the liver and kidney. Most CSF longitudinal studies begin with the MCI diagnosis [8, 9], and the predictive powder of CSF Aβ levels at the pre-MCI stage has not been fully investigated [18, 55, 56].

Our systematic review suggests a model of differential longitudinal changes in plasma Aβ levels in cognitively stable individuals versus those who go on to develop AD dementia. This model is depicted in Figure 4. In the cognitively stable, plasma Aβ levels gradually increase over time by a modest amount [26]. Our data suggest that cognitively normal subjects with higher initial Aβ levels may be at greater risk of progression to AD, and that $A\beta_{1-42}$ levels then tend to decrease later, probably as Aβ deposition commences prior to diagnosis. In this AD converter group, plasma Aβ levels are therefore initially elevated, reach a pre-diagnosis inflection point, and then eventually fall to lie in the same range as normal controls, or even marginally below [57]. Our model is therefore consistent with a pathophysiological AD process that starts years before even the pre-dementia phase of MCI [58]. High plasma Aβ levels may reflect a genetic or other predisposition to increased production, or reduced clearance of Aβ [40]. Cognitively normal subjects with high basal Aβ levels may therefore have a higher risk of developing AD than matched peers with low Aβ levels. One recent meta-analytic study reported that increased $A\beta_{1-40}$ levels showed a weak association with conversion from MCI to AD (Cohen's delta 0.18, $p = 0.15$) [29], which is also consistent with our model. Hypothetically, the reduction of soluble $A\beta_{1-42}$ in later stages may result from a "sink" effect of amyloid plaque and vascular deposition, preventing transport of $A\beta_{1-42}$ from the brain to the plasma via the interstitial fluid [52, 59, 60]. We cannot, however, conclude whether high baseline plasma Aβ is a clinically useful prognostic AD biomarker until further longitudinal studies provide more information about sensitivity, specificity, and positive and negative predictive value. Future studies may wish to focus on whether evidence of an inflection point on repeat plasma testing is particularly sensitive to future development of dementia.

In conclusion, results of this meta-analysis suggest that higher basal $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in cognitively normal elderly individuals might be predictors of higher rates of progression to AD or dementia. The trends in the cross-sectional data suggest lower $A\beta_{1-42}$ levels once progression to clinically diagnosed AD has occurred. These data might reflect the neurochemistry of $A\beta_{1-42}$, with initially higher peptide levels generated by increased

expression or cleavage, progressing to lower levels possibly due to plaque deposition in the later stages of the disease. Further research is required to establish the patient-specific clinical utility of these potentially prognostic blood-based biomarkers of AD.

Acknowledgments

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A   Aβ$_{1-40}$ levels

Study	N	AD Mean (SD)	N	Control Mean (SD)	WMD (random) 95% CI	Weight %	WMD (random) 95% CI
Tamaoka 1996	28	305.20(181.90)	25	296.50(91.20)		3.83	8.70 [-67.57, 84.97]
Kosaka 1997	44	297.00(47.60)	15	335.50(42.40)		12.91	-38.50 [-64.16, -12.84]
Arvanitakis 2002	220	984.80(1042.30)	59	774.70(722.40)		0.52	210.10 [-20.00, 440.20]
Fukumoto 2003	146	226.50(75.70)	92	211.40(74.40)		14.79	15.10 [-4.44, 34.64]
Sobow 2005	54	168.70(32.20)	35	160.10(15.20)		17.38	8.60 [-1.36, 18.56]
Kulstad 2006	59	567.30(268.07)	50	502.70(248.90)		2.56	64.60 [-32.55, 161.75]
Abdullah 2007	67	103.38(34.95)	146	80.66(22.47)		17.56	22.72 [13.59, 31.85]
Fagan 2007	16	214.00(93.00)	65	191.00(61.30)		7.36	23.00 [-24.94, 70.94]
Giedraitis 2007	39	260.20(56.60)	18	280.10(73.50)		9.41	-19.90 [-58.22, 18.42]
Buerger 2009	17	166.00(42.00)	15	208.00(52.00)		10.77	-42.00 [-75.03, -8.97]
Roher 2009	17	424.06(147.73)	21	344.41(132.43)		2.91	79.65 [-10.57, 169.87]
Total (95% CI)	707		541			100.00	3.43 [-13.33, 20.19]

Test for overall effect: Z = 0.40 (P = 0.69)

-100   -50   0   50   100
Control   AD

B   Aβ$_{1-42}$ levels

Study	N	AD Mean (SD)	N	control Mean (SD)	WMD (random) 95% CI	Weight %	WMD (random) 95% CI
Tamaoka 1996	28	276.70(182.80)	25	194.60(115.10)		0.16	82.10 [0.74, 163.46]
Kosaka 1997	44	44.20(14.90)	15	48.30(9.50)		11.31	-4.10 [-10.62, 2.42]
Arvanitakis 2002	220	92.50(106.10)	59	85.30(88.50)		1.37	7.20 [-19.38, 33.78]
Fukumoto 2003	146	33.40(24.40)	92	31.60(14.00)		14.20	1.80 [-3.08, 6.68]
Sobow 2005	54	37.80(10.30)	35	36.30(6.30)		16.99	1.50 [-1.95, 4.95]
Kulstad 2006	59	31.20(25.35)	50	41.80(25.46)		7.35	-10.60 [-20.17, -1.03]
Pesaresi 2006	146	38.05(13.92)	89	52.30(29.59)		11.26	-14.25 [-20.80, -7.70]
Abdullah 2007	67	4.53(4.26)	146	5.66(3.75)		20.53	-1.13 [-2.32, 0.06]
Fagan 2007	16	36.00(37.20)	65	36.00(29.40)		2.39	0.00 [-19.58, 19.58]
Giedraitis 2007	39	97.50(86.20)	18	114.70(124.60)		0.25	-17.20 [-80.80, 46.40]
Buerger 2009	17	20.00(8.00)	15	24.00(7.00)		13.61	-4.00 [-9.20, 1.20]
Roher 2009	17	139.91(77.82)	21	124.71(42.34)		0.59	15.20 [-25.99, 56.39]
Total (95% CI)	853		630			100.00	-2.84 [-6.06, 0.38]

Test for overall effect: Z = 1.73 (P = 0.08)
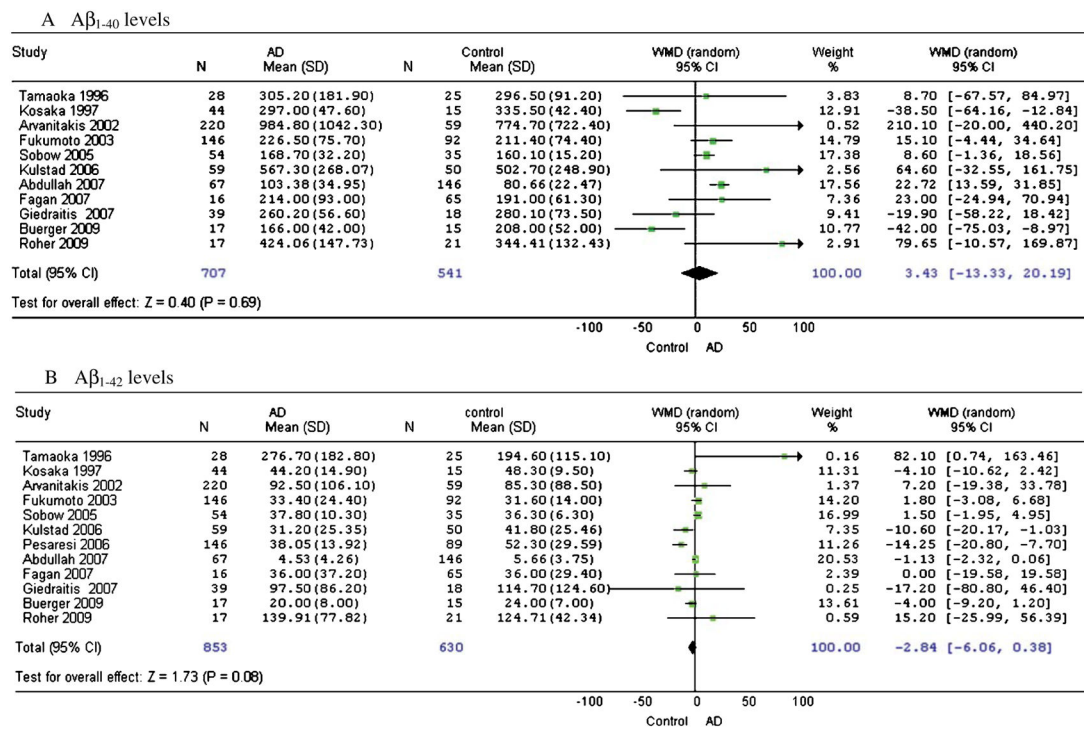
-100   -50   0   50   100
Control   AD

Fig. 1.

Forest plots for plasma Aβ$_{1-40}$ and Aβ$_{1-42}$ levels in AD and cognitively normal control subjects in cross-sectional studies.

A Aβ$_{1-40}$ levels

Study or sub-category	N	Incident AD Mean (SD)	N	Remaining normal Mean (SD)	WMD (random) 95% CI	Weight %	WMD (random) 95% CI
Mayeux 1999	64	134.70 (46.40)	105	111.80 (44.06)		12.50	22.90 [8.75, 37.05]
Mayeux 2003	86	136.20 (46.70)	365	133.30 (61.90)		17.28	2.90 [-8.84, 14.64]
Lopez 2008	55	161.40 (55.60)	117	142.10 (59.70)		7.88	19.30 [1.05, 37.55]
Schupf 2008	104	87.20 (55.07)	1021	79.60 (60.70)		18.63	7.60 [-3.62, 18.82]
Lambert 2009	154	241.80 (50.90)	985	233.30 (49.90)		28.21	8.50 [-0.12, 17.12]
Cosentino 2010	70	73.41 (50.69)	481	63.11 (43.97)		15.50	10.30 [-2.21, 22.81]
Total (95% CI)	533		3074			100.00	10.29 [4.98, 15.61]

Test for heterogeneity: Chi² = 5.89, df = 5 (P = 0.32), I² = 15.1%
Test for overall effect: Z = 3.80 (P = 0.0001)

-100 -50 0 50 100
Remaining normal   Incident AD

B Aβ$_{1-42}$ levels

Study or sub-category	N	Incident AD Mean (SD)	N	Remaining normal Mean (SD)	WMD (random) 95% CI	Weight %	WMD (random) 95% CI
Mayeux 1999	64	82.40 (68.80)	105	51.50 (42.01)		6.63	30.90 [12.23, 49.57]
Mayeux 2003	86	76.50 (59.80)	365	58.80 (32.90)		10.42	17.70 [4.62, 30.78]
Blasko 2008	46	68.39 (157.08)	267	33.41 (65.43)		1.43	34.98 [-11.09, 81.05]
Lopez 2008	55	24.50 (16.00)	117	17.30 (13.40)		19.86	7.20 [2.32, 12.08]
Schupf 2008	104	42.20 (29.57)	1021	37.30 (24.60)		18.62	4.90 [-0.98, 10.78]
Lambert 2009	154	38.70 (9.80)	985	39.30 (9.80)		22.80	-0.60 [-2.26, 1.06]
Cosentino 2010	70	37.59 (18.16)	481	30.62 (18.02)		20.24	6.97 [2.42, 11.52]
Total (95% CI)	579		3341			100.00	8.01 [2.32, 13.70]

Test for heterogeneity: Chi² = 35.64, df = 6 (P < 0.00001), I² = 83.2%
Test for overall effect: Z = 2.76 (P = 0.006)

-100 -50 0 50 100
Remaining normal   Incident AD

C Aβ$_{1-42}$/Aβ$_{1-40}$ ratios

Study or sub-category	N	Incident AD Mean (SD)	N	Remaining normal Mean (SD)	WMD (random) 95% CI	Weight %	WMD (random) 95% CI
Mayeux 1999	64	0.67 (0.56)	105	0.51 (0.41)		5.44	0.16 [0.00, 0.32]
Mayeux 2003	86	0.61 (0.53)	365	0.48 (0.30)		8.99	0.13 [0.01, 0.25]
Lopez 2008	55	0.14 (0.07)	117	0.11 (0.06)		34.63	0.03 [0.01, 0.05]
Schupf 2008	104	0.64 (0.61)	1021	0.60 (0.32)		8.68	0.04 [-0.08, 0.16]
Lambert 2009	154	0.16 (0.04)	985	0.17 (0.05)		38.05	-0.01 [-0.02, 0.00]
Cosentino 2010	70	0.70 (0.76)	481	0.63 (0.48)		4.21	0.07 [-0.11, 0.25]
Total (95% CI)	533		3074			100.00	0.03 [-0.01, 0.07]

Test for heterogeneity: Chi² = 22.72, df = 5 (P = 0.0004), I² = 78.0%
Test for overall effect: Z = 1.65 (P = 0.10)

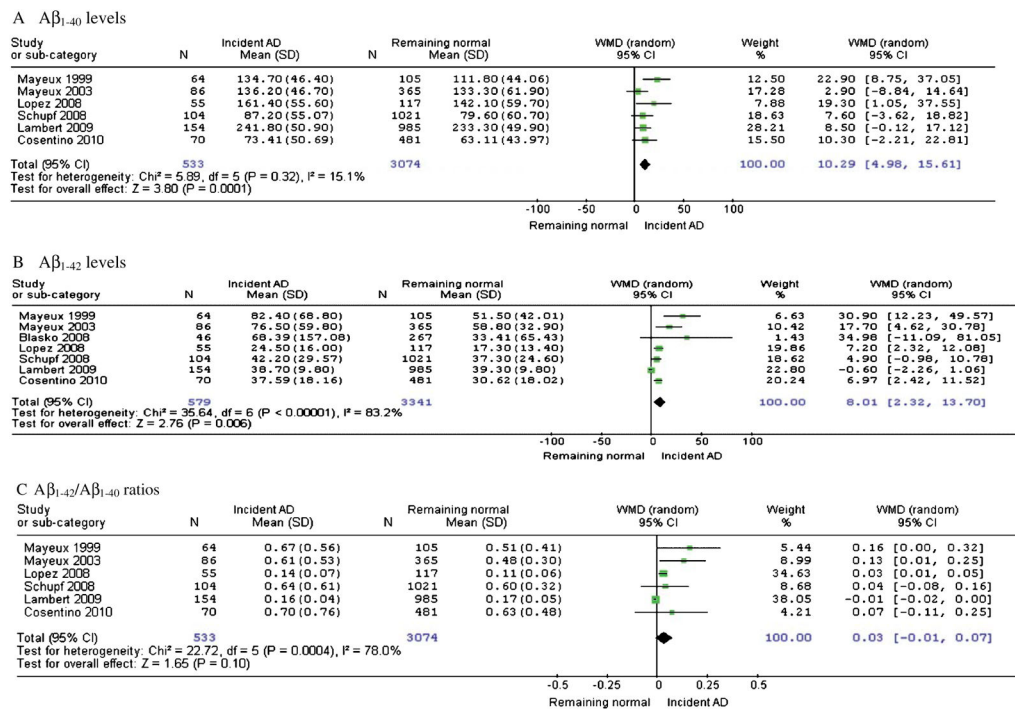-0.5 -0.25 0 0.25 0.5
Remaining normal   Incident AD

Fig. 2.

Forest plots for baseline plasma Aβ levels for incident AD subjects compared to those who remain normal upon follow up.
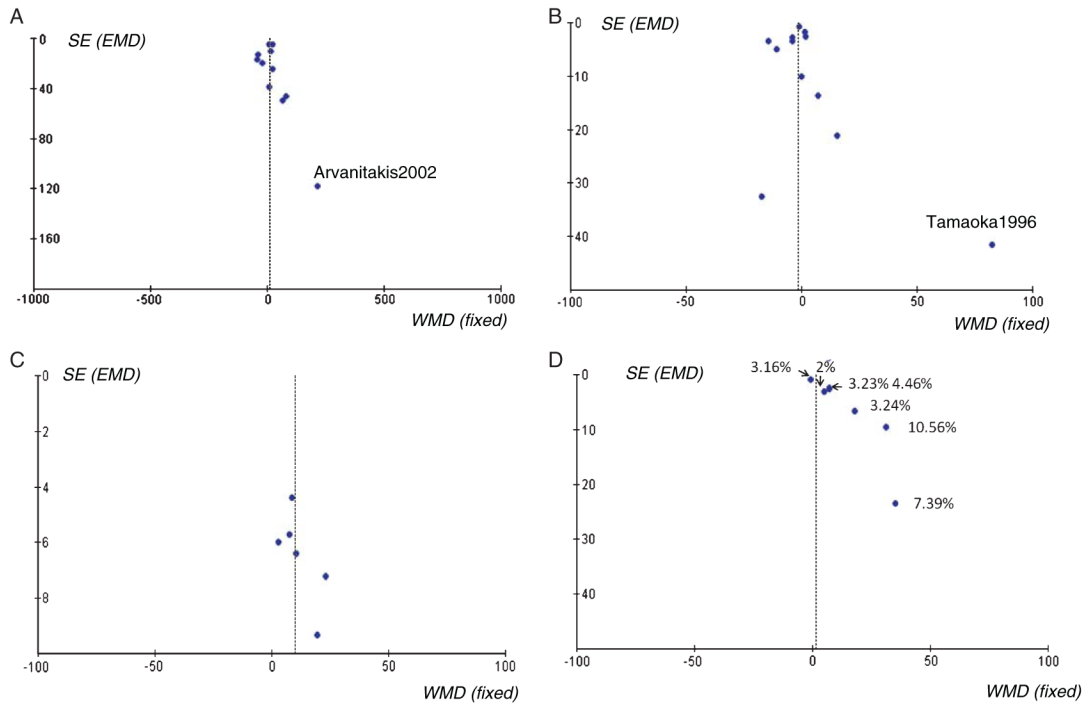
Fig. 3.
Funnel plots for plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in cross-sectional and longitudinal studies. Fig. 3A. Plasma $A\beta_{1-40}$ levels in AD and cognitively normal control subjects in cross-sectional studies. Fig. 3B. Plasma $A\beta_{1-42}$ levels in AD and cognitively normal control subjects in cross-sectional studies. Fig. 3C. Baseline plasma $A\beta_{1-40}$ levels for incident AD and non-dementia normal groups upon follow up. Fig. 3D. Baseline plasma $A\beta_{1-42}$ levels for incident AD and non-dementia normal groups upon follow up. The labeled numbers are annual conversion rates from non-dementia to AD for each study. (Conversion rate = incident AD number/total participants).
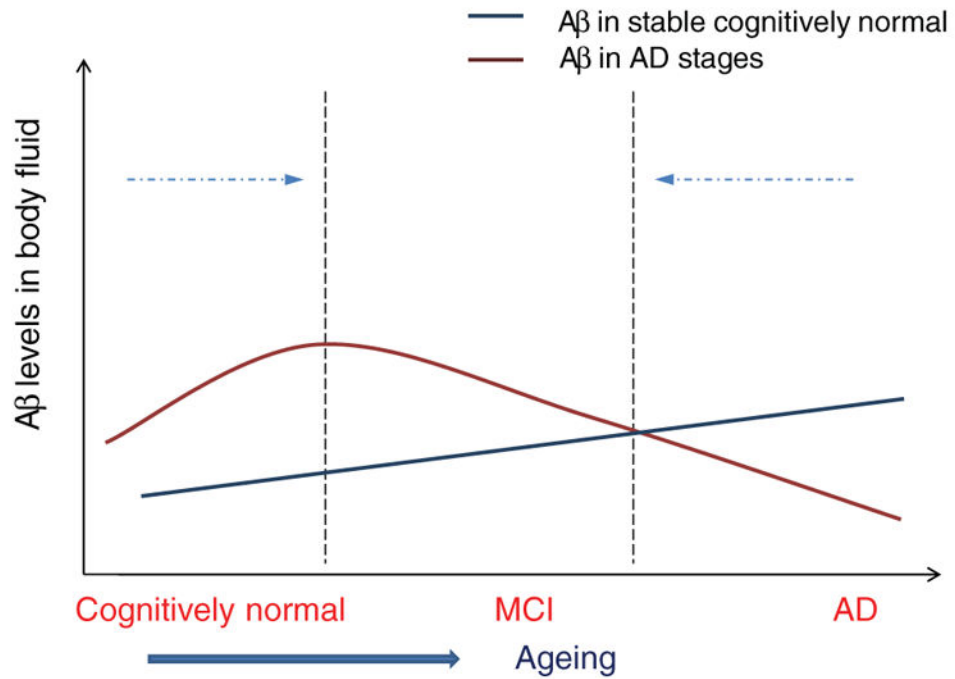
Fig. 4.
A model for plasma $A\beta_{1-42}$ level changes in AD and normal aging. Evidence suggests that $A\beta$ levels in cognitively stable individuals increase slightly with age. In those who eventually develop clinical AD, $A\beta_{1-42}$ levels are elevated in the pre-dementia stage, reach a peak, and then diminish prior to the development of clinical AD symptoms. Hence, when comparing plasma levels crosssection-ally between AD patients and controls, these will tend to converge.

Table 1

Cross sectional studies used for the meta-analysis

Study	Diagnosis (n)	Age	APOE ε4 (%)	Plasma Aβ$_{1-40}$ (pg/ml)	Plasma Aβ$_{1-42}$ (pg/ml)	Aβ$_{1-42/1-40}$	Aβ$_{1-40/1-42}$	Methods	Fasting type (anticoagulation, storage)
Buerger 2009 [15]	Control (15)	70.2 ± 10.6¶	46.7	208 ± 52	24 ± 7			Aβ$_{1-40}$ (ELISA, The Genetics Company, Switzerland)	N/A (EDTA, −80°C)
	AD (17)		52.9	166 ± 42	20 ± 8			Aβ$_{1-42}$ (ELISA, Innogenetics, Ghent, Belgium)	
Roher 2009 [11]	Control (21)	75.8 ± 7.1	47.6	344.41 ± 132.43	124.71 ± 42.34	0.44 ± 0.30		Aβ$_{1-40}$ (ELISA, Immunobiological Laboratories, Minneapolis, MN)	Fasting (N/A)
	AD (17)	81.4 ± 5.3	64.7	424.06 ± 147.73	139.91 ± 77.82	0.16 ± 0.29		Aβ$_{1-42}$ (ELISA, Innogenetics, Ghent, Belgium)	
Giedraitis 2007 [16]	Control (18)	65.9 ± 8.6	27.8	280.1 ± 73.5*	114.7 ± 124.6*			Aβ$_{1-40}$ (antibody:BNT77/ BA27)	N/A (heparin, −80°C)
	AD (39)	65.9 ± 7.9	44.9	260.2 ± 56.6*	97.5 ± 86.2*			Aβ$_{1-42}$ (antibody:BNT77/BC05) (ELISA, Takeda Pharmaceuticals, Osaka, Japan)	
Abdullah 2007 [17]	Control (146)	74.15 ± 0.69	N/A	80.66 ± 22.47†	5.66 ± 3.75†	0.06 ± 0.006†		Aβ$_{1-40}$, Aβ$_{1-42}$ (ELISA, Invitrogen, CA, USA)	N/A (EDTA, −80°C)
	AD (67)	76.16 ± 0.96		103.38 ± 34.95†	4.53 ± 4.26†	0.05 ± 0.008†			
Fagan 2007 [18]	Control (65)	73.3 ± 8.4	32	191 ± 61.3	36 ± 29.4		8.64 ± 8.9	Aβ$_{1-40}$ (antibody: m266/m2G3, ELISA)€	Fasting (EDTA, −84°C)
	AD (16)	75.2 ± 5.8	37	214 ± 90.3	36 ± 37.2		9.25 ± 7.0	Aβ$_{1-42}$ (antibody: m266/m21F12, ELISA)	
Kulstad 2006 [19]	Control (50)	70.5 ± 1.1	N/A	502.7 ± 248.9†	41.8 ± 25.46†			Aβ$_{1-40}$, Aβ$_{1-42}$(ELISA, Signet Laboratories, Dedham, MA)	Fasting (N/A)
	AD (59)	71.4 ± 1.0		567.3 ± 268.1†	31.2 ± 25.35†				
Sobow 2005 [20]	Control (35)	75.0 ± 2.9	N/A	160.1 ± 15.2	36.3 ± 6.3		4.5 ± 0.6	Aβ$_{1-40}$, Aβ$_{1-42}$(ELISA, BioSource Intl, Inc)	Fasting (EDTA, −70°C)
	AD (54)	77.5 ± 4.4		168.7 ± 32.2	37.8 ± 10.3		4.6 ± 0.9		
Fukumoto 2003 [21]	Control (92)	69.4 ± 10.3	1.1	211.4 ± 74.4*	31.6 ± 14.0*			Aβ$_{1-40}$(antibody:BNT77/ BA27)	N/A (EDTA, −80°C)
	AD (146)	76.0 ± 8.2	3.8	226.5 ± 75.7*	33.4 ± 24.4*			Aβ$_{1-42}$(antibody:BNT77/BC05) (ELISA, Takeda Pharmaceuticals, Osaka,Japan)	
Arvanitakis 2002 [22]	Control (59)	74.9 ± 7.8	N/A	774.7 ± 722.4*	85.3 ± 88.5*			Aβ$_{1-40}$(antibody:BAN50/BA27, ELISA)€	N/A
	AD (220)	77.7 ± 7.6		984.8 ± 1042.3*	92.5 ± 106.1*			Aβ$_{1-42}$ (antibody:BAN50/BC05, ELISA)	
Kosaka 1997 [23]	Control (15)	72.3	N/A	335.5 ± 42.4*	48.3 ± 9.5*			Aβ$_{1-40}$(antibody:BNT77/ BA27, ELISA)€	N/A (EDTA, −80°C)
	AD (44)	71.9		297.0 ± 47.6*	44.2 ± 14.9*			Aβ$_{1-42}$(antibody:BNT77/BC05, ELISA)	
Tamaoka 1996 [24]	Control (25)	64.5 ± 9.20	N/A	296.5 ± 91.2*	194.6 ± 115.1*			Aβ$_{1-40}$(antibody:BAN50/BA27, ELISA)€	Fasting (Sodium azide & PMSF/ −20°C)
	AD (28)	73.8 ± 8.97		305.2 ± 181.9*	276.7 ± 182.8*			Aβ$_{1-42}$ (antibody:BAN50/BC05, ELISA)	
Pesaresi 2006 [13]	Control (89)	68.23 ± 12.08	5.5		52.30 ± 29.59**			INNOTEST Aβ$_{1-42}$ (ELISA, Imogenetics, Ghent, Belgium)	N/A (EDTA, −80°C)

Study	Diagnosis (n)	Age	APOE ε4 (%)	Plasma Aβ₁₋₄₀ (pg/ml)	Plasma Aβ₁₋₄₂ (pg/ml)	Aβ₁₋₄₂/₁₋₄₀	Aβ₁₋₄₀/₁₋₄₂	Methods	Fasting type (anticoagulation, storage)
	AD (146)	73.76 ± 7.62	29.09		38.05 ± 13.92 **				

Data are presented as Mean ± SD;

*
For ease of comparison all Aβ values were expressed in pg/mL. pM or pmol/L values were converted using molecular weight values of 4323 g/mol for Aβ1–40 and 4514 g/mol for Aβ1–42;

†
SD (standard deviation) was calculated from SE (standard error);

**
Mean and SD values were obtained from corresponding authors at our request;

€
No commercial information is presented about antibodies in methods of articles; N/A The relevant information is not published in articles;

¶
mean age for the whole study; EDTA, Ethylenediaminetetraacetic acid; PMSF, phenylmethylsulfonyl fluoride.

Table 2

Characteristics of the longitudinal studies used for meta-analysis

Study	Follow-up months	Diagnosis (n)	Baseline age	APOE e4 (%)	Baseline $A\beta_{1-40}$ (pg/ml)	Baseline $A\beta_{1-42}$ (pg/ml)	Baseline $A\beta_{1-42}/A\beta_{1-40}$	Methods	Fasting type (anticoagulation, storage)
Cosentino 2010 [28]	54	Normal-to-AD (70)	80.84 ± 6.98	29	73.41 ± 50.69	37.59 ± 18.16	0.70 ± 0.76	$A\beta_{1-40}$ (6E10/R162 antisera, ELISA)€	N/A (EDTA, N/A)
		Remaining normal (481)	74.52 ± 5.37	26	63.11 ± 43.97	0.62 ± 18.02	0.63 ± 0.48	$A\beta_{1-42}$ (6E10/R165 antisera, ELISA)	
Lambert 2009 [10]	48	Normal-to-Dementia (233)//	77.9 ± 5.6	18.2	243.7 ± 51.9	38.8 ± 9.6	0.163 ± 0.041	INNO-BIA multiplex kit (Innogenetics, Ghent, Belgium)	Non-Fasting (EDTA, −80°C)
		Normal-to-AD (154)**			241.8 ± 50.9**	38.7 ± 9.8**	0.163 ± 0.041**		
		Remaining normal (985)	73.8 ± 5.3	10.4	233.3 ± 49.9	39.3 ± 9.8	0.173 ± 0.046		
Schupf 2008 [25]	55.2	Normal-to-AD (104)	80.7 ± 7.0*	27.2	87.2 ± 55.07*	42.2 ± 29.57*	0.64 ± 0.61*	$A\beta_{1-40}$ (6E10/R162 antisera, ELISA) €	N/A (EDTA, N/A)
		Remaining normal (1021)	76.3 ± 6.1*	26.3	79.6 ± 60.7*	37.3 ± 24.60*	0.6 ± 0.32*	$A\beta_{1-42}$ (6E10/R165 antisera, ELISA)	
Lopez 2008 [26]	54	Normal-to-AD (55)	80.2 ± 3.6	31	161.4 ± 55.6	24.5 ± 16.0	0.14 ± 0.07	6E10/antisera (ELISA, Ana Spec, San Jose, CA)	Fasting (EDTA, −70°C)
		Remaining normal (117)	78.6 ± 3.6	20	142.1 ± 59.7	17.3 ± 13.4	0.11 ± 0.06		
Blasko 2008 [12]	30	Normal-to-AD (55)		N/A		68.39 ± 157.08**		INNOTEST (ELISA Innogenetics, Ghent, Belgium)	N/A (EDTA, −80°C)
		Remaining normal (117)		N/A		33.41 ± 65.43**			
Mayeux 2003† [14]	60	Normal-to-AD (86)	79.3 ± 6.6	32.1	136.2 ± 46.7	76.5 ± 59.8	0.61 ± 0.53	$A\beta_{1-40}$ (6E10/R162 antisera, ELISA) €	N/A (N/A, −70°C)
		Remaining normal (365)	75.5 ± 5.9	26.7	133.3 ± 61.9	58.8 ± 32.9	0.48 ± 0.3	$A\beta_{1-42}$ (6E10/R165 antisera, ELISA)	
Mayeux 1999† [27]	43.2	Normal-to-AD (64)	77.4 ± 5.9	N/A	134.70 ± 46.40*	82.4 ± 68.8*	0.67 ± 0.56*	$A\beta_{1-40}$ (6E10/R162 antisera, ELISA)€	N/A (N/A, −70°C)
		Remaining normal (105)	73.4 ± 5.3	N/A	111.8 ± 44.06*	51.5 ± 42.01*	0.51 ± 0.41*	$A\beta_{1-42}$ (6E10/R165 antisera, ELISA)	

Data are presented as Mean ± SD;

* SD (standard deviation) was calculated from SE (standard error).

† Authors confirmed that there were no overlapping data in studies of Mayeux 1999 and Mayeux 2003.

// In the study of Lambert 2009, 233 incident dementia patients included 154 AD, 46 with mixed or pure vascular dementia, and 33 other dementia.

** Mean and/or SD values were obtained from corresponding authors; N/A relevant information is not presented by authors. EDTA, Ethylenediaminetetraacetic acid.