

Association of plasma amyloid β with risk of dementia

The prospective Three-City Study

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ABSTRACT

Objective: Several lines of evidence indicate that a decrease in the CSF concentration of amyloid β_{42} ($A\beta_{42}$) is a potential biomarker for incident Alzheimer disease. In contrast, studies on plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ peptide levels have yielded contradictory results. Here, we explored the links between incident dementia and plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ peptide concentrations in the prospective, population-based Three-City (3C) Study. We also assessed the association between plasma concentrations of truncated $A\beta$ ($A\beta_{n-40}$ and $A\beta_{n-42}$) and the risk of dementia.

Methods: During a subsequent 4-year follow-up period, 257 individuals presented incident dementia from 8,414 participants, and a subcohort of 1,185 individuals without dementia was drawn as a control cohort. Plasma levels of $A\beta_{1-40}$, $A\beta_{1-42}$, $A\beta_{n-40}$, and $A\beta_{n-42}$ were measured using an xMAP-based assay technology. The association between plasma $A\beta$ peptide levels and the risk of dementia was assessed using Cox proportional hazard models.

Results: Of the various $A\beta$ variables analyzed, the $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-42}/A\beta_{n-40}$ ratios presented the strongest association with the risk of dementia: people with a high $A\beta_{1-42}/A\beta_{1-40}$ or $A\beta_{n-42}/A\beta_{n-40}$ ratio had a lower risk of developing dementia. These associations were restricted to individuals diagnosed at 2 years of follow-up and the $A\beta_{n-42}/A\beta_{n-40}$ ratio was mainly associated with the risk of mixed/vascular dementia.

Conclusion: Plasma $A\beta$ peptide concentrations and $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-42}/A\beta_{n-40}$ ratios may be useful markers to indicate individuals susceptible to short-term risk of dementia.

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GLOSSARY

$A\beta$ = amyloid β ; **AD** = Alzheimer disease; **BMI** = body mass index; **CI** = confidence interval; **DSM-IV** = *Diagnostic and Statistical Manual of Mental Disorders, 4th edition*; **HDL** = high-density lipoprotein; **HR** = hazard ratio; **INSERM** = Institut National de la Santé et de la Recherche Médicale; **MCI** = mild cognitive impairment; **3C** = Three-City.

Many studies have shown that low CSF levels of the 42-amino acid amyloid β fragment ($A\beta_{42}$) are strongly associated with current Alzheimer disease (AD) or the future development of AD in patients with mild cognitive impairment (MCI). When combined with CSF tau protein assays, this tool is now starting to be used in clinical diagnostic support.^{1,2} However, because CSF sampling is commonly regarded as an invasive and time-consuming procedure, there has been increasing interest in establishing whether the quantification of plasma levels of $A\beta$ peptides is also relevant.

However, the results in this respect are contradictory; in people with monogenic forms of AD, plasma concentrations of both $A\beta_{1-40}$ and $A\beta_{1-42}$ may be increased,³ whereas in sporadic cases, studies have variously reported unchanged levels of plasma $A\beta$, increased levels of plasma $A\beta_{1-42}$ and/or $A\beta_{1-40}$, and low levels of plasma $A\beta_{1-42}$.⁴⁻¹³

Given this background, we attempted to evaluate the relationship between the risk of incident dementia on one hand and plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations on the other in the Three-City (3C) Study, a prospective, population-based cohort study of men and women aged

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65 years and older. We also assessed the association between plasma levels of truncated A β peptides (A β_{n-40} and A β_{n-42}) and the risk of developing incident dementia in a large population-based cohort for the first time, to the best of our knowledge. Truncated A β species represent more than 60% of all A β species at the earliest stage of Alzheimer pathology.¹⁴ These truncated species have been shown to induce learning impairment and neuronal apoptosis when injected in mouse models.¹⁵ Furthermore, it has been suggested that CSF levels of these truncated peptides are of value in the diagnosis of AD.¹⁶

METHODS The 3C Study is a population-based, prospective study of the relationship between vascular factors and dementia.¹⁷ It has been conducted in 3 French cities: Bordeaux (southwest France), Montpellier (south France), and Dijon (central eastern France). A sample of noninstitutionalized subjects older than 65 years was randomly selected from the electoral rolls of each city. Between January 1999 and March 2001, 9,686 subjects meeting the inclusion criteria agreed to participate. After recruitment, 392 subjects withdrew from the study. Thus, 9,294 subjects were finally included in the study (2,104 in Bordeaux, 4,931 in Dijon, and 2,259 in Montpellier). All subjects or, in those with substantial cognitive impairment, a caregiver, legal guardian, or other proxy gave written informed consent for participation in this study. The study protocols were reviewed and approved by the Ethical Committee of the University Hospital of Kremlin-Bicêtre.

At the baseline clinical examination, blood samples were obtained from 8,414 individuals who were representative of the source population. Follow-up examinations were performed in 2001–2003 and 2003–2005. During the follow-up period, 257 individuals developed incident dementia. Dementia was diagnosed using a 3-step procedure.¹⁸ Trained psychologists administered a battery of neuropsychological tests. Next, all participants in Bordeaux and Montpellier were examined by a neurologist at baseline, whereas in Dijon, only those who screened positive for dementia underwent further examination (because of the large number of participants in that center). During follow-up, participants with suspected incident dementia (on the basis of their neuropsychological test results) were examined by a neurologist. Last, an independent committee of neurologists reviewed all potential prevalent and incident cases of dementia to obtain a consensus on the condition's diagnosis and etiology, according to the criteria given in the *DSM-IV*. Dementia classification was based on the National Institute of Neurological and Communication Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria for AD and the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria for vascular dementia.^{19–21} Subjects with a typical history of AD (progressive worsening of memory or other cognitive functions) and documented stroke were classified as having mixed dementia. Diagnosis was supported by MRI or CT examination when available. To ensure sufficient power, mixed dementia and pure vascular dementia were pooled (27 mixed dementia and 24 pure vascular dementia).

A case-cohort study was built at the end of the 4-year follow-up period, to investigate potential, novel risk markers for dementia (figure e-1 on the *Neurology*[®] Web site at www.neurology.org). In the present work, the study population was based on a subcohort of 1,254 subjects randomly selected from the source sample totaling 8,414 individuals (i.e., a sampling ratio of 15%) stratified by center, 5-year age class, and sex. Twenty-nine subcohort subjects were diagnosed as having dementia at baseline and were thus excluded from our analysis. Incident dementia was diagnosed in 40 subjects included in the subcohort. We then added 217 subjects with dementia from outside the subcohort, giving a total of 257 cases of incident dementia: 167 cases of AD, 51 cases of mixed/vascular dementia, 15 cases of dementia with parkinsonism, and 24 cases of other types of dementia (Lewy body dementia: 8; other dementias [associated with multiple sclerosis, alcoholism, etc.]: 11; undefined dementia: 5).

In the subcohort, 121 individuals were excluded because of a lack of information from baseline on their dementia status (death or lost of follow-up). Individuals for whom at least 1 A β plasma concentration (n = 64) or covariable (n = 4) measurement was missing were excluded, together with individuals exhibiting at least 1 aberrant A β plasma concentration measurement (n = 11). These selection steps allowed us to define a control cohort of 985 individuals.

Cases with dementia for whom at least 1 A β plasma concentration (n = 11) or covariable (n = 10) was missing were also excluded, together with cases exhibiting at least 1 aberrant A β plasma concentration measurement (n = 3). These selection steps enabled us to obtain 233 cases of incident dementia (121 diagnosed at 2 years of follow-up and 112 at 4 years): 154 cases of AD, 46 cases of mixed or vascular dementia, 33 cases of other types of dementia, 8 other dementias, and 11 undefined dementia.

The main baseline characteristics of the source sample, the control cohort, and the incident dementia population are shown in table 1.

Nonfasting plasma samples were collected in tubes containing salt ethylenediaminetetra-acetic acid as an anticoagulant at baseline. After centrifugation, plasma samples were divided into aliquots in polypropylene tubes, stored at -80°C , and only thawed immediately before A β quantification. The plasma A β peptide assay was performed using an INNO-BIA kit (Innogenetics, Ghent, Belgium) based on a multiplex xMAP (Luminex, Austin, TX) technique. A β_{1-40} and A β_{1-42} (format A) and A β_{n-40} and A β_{n-42} (format B) were simultaneously analyzed.⁷ At this level, it is important to note that the affinity of antibodies used in format A (3D6) and B (4G8) for A β are different, and it makes it difficult to compare the absolute levels of the full-length forms with the truncated forms. No A β plasma measurements were available for 6 individuals in the control cohort and 3 in the population with dementia.

The data were analyzed statistically using SAS software (release 9.1, SAS Institute Inc., Cary, NC). Covariables for adjustments were selected as described in appendix e-1. Cases with dementia and controls were compared in terms of plasma A β_{1-40} , A β_{1-42} , A β_{n-40} , A β_{n-42} , A $\beta_{1-42}/\text{A}\beta_{1-40}$, and A $\beta_{n-42}/\text{A}\beta_{n-40}$ concentrations in an analysis of covariance (ANCOVA) that adopted a general linear model (GLM procedure) adjusted (or not) for age, center, sex, educational level, diabetes, high-density lipoprotein (HDL) cholesterol, body mass index (BMI), and the presence or absence of the APOE $\epsilon 4$ allele. A proportional hazards model with delayed entry, with age as the time scale, and weighted according to the reciprocal of the sampled fraction of the source population, as previously described,²¹ was used to estimate the association between plasma A β concentra-

Table 1 Baseline sociodemographic variables and potential confounding vascular risk factors for the source population, control cohort, and incident dementia population (see Methods for details)

	Source population (n = 8,414)	Control cohort (n = 985)	Incident dementia (n = 233)
Age, y (mean ± SD)	74.2 ± 5.5	73.8 ± 5.3	77.9 ± 5.6
Women, %	60.4	61.3	58.4
ε4 allele frequency, %	10.5	10.4	18.2
BMI, kg/m ² (mean ± SD)	25.7 ± 4.0	25.7 ± 4.1	25.0 ± 4.0
HDL cholesterol, mmol/L (mean ± SD)	1.63 ± 0.42	1.63 ± 0.40	1.57 ± 0.38
Diabetes mellitus, %	9.8	8.6	17.0
Low educational level,* %	47.5	46.6	57.1
Months from inclusion to development of dementia			33.0 (11.7)

*Low educational level = primary education without a diploma, primary education with a diploma, secondary education without a baccalaureate degree.
BMI = body mass index; HDL = high-density lipoprotein.

tions and the risk of dementia. We analyzed the association of $A\beta_{1-40}$, $A\beta_{1-42}$, $A\beta_{n-40}$, $A\beta_{n-42}$, $A\beta_{1-42}/A\beta_{1-40}$, and $A\beta_{n-42}/A\beta_{n-40}$ with the risk of dementia by entering these variables as linear terms (per SD). Furthermore, tertiles were defined (table e-1) and the lowest was used as a reference. Hazard ratios were systematically adjusted for center, age, sex, and educational level (model 1). Analyses were subsequently adjusted for other vascular confounders, as defined above: diabetes, HDL cholesterol, BMI, and the presence or absence of the APOE ε4 allele (model 2). Similarly, we analyzed the association between plasma $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-42}/A\beta_{n-40}$ levels on one hand and the risk of incidence of the various dementia subtypes on the other.

RESULTS Individuals who developed dementia during the follow-up period had significantly higher baseline concentrations of plasma $A\beta_{1-40}$ and $A\beta_{n-40}$ and significantly lower $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-42}/A\beta_{n-40}$ ratios when compared with controls. However, only associations with low $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-42}/A\beta_{n-40}$ ratios remained significant after adjustment for covariables (table 2).

A high $A\beta_{1-42}/A\beta_{1-40}$ ratio was associated with a lower risk of developing dementia, whatever the statistical model used; individuals in the upper tertile

Table 2 Plasma Aβ peptide levels and the risk of dementia: Values at baseline (mean ± SD)

	No dementia (n = 985)	Dementia (n = 233)	Crude p value	p (model 2)
$A\beta_{1-40}$ (pg/mL)	233.3 ± 49.9	243.7 ± 51.9	0.005	0.23
$A\beta_{1-42}$ (pg/mL)	39.3 ± 9.8	38.8 ± 9.6	0.48	0.16
$A\beta_{1-42}/A\beta_{1-40}$	0.173 ± 0.046	0.163 ± 0.041	0.004	0.04
$A\beta_{n-40}$ (pg/mL)	243.8 ± 52.9	258.8 ± 58.0	0.0001	0.11
$A\beta_{n-42}$ (pg/mL)	26.8 ± 7.7	27.1 ± 7.5	0.70	0.40
$A\beta_{n-42}/A\beta_{n-40}$	0.113 ± 0.036	0.107 ± 0.029	0.02	0.04

Model 2: adjusted for center, age, gender, educational level, high-density lipoprotein cholesterol, body mass index, diabetes, and APOE genotype.

had a 2-fold lower risk of developing dementia (tables 3 and e-2). A similar result was obtained when analyzing the $A\beta_{n-42}/A\beta_{n-40}$ ratio, although the effect was weaker (table 3). Importantly, high $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-42}/A\beta_{n-40}$ ratios were significantly associated with a lower risk of developing dementia only in individuals diagnosed at 2 years of follow-up (table e-3).

Because the association with the ratio of $A\beta_{1-40}$ and $A\beta_{1-42}$ was highly significant, we looked at their combined effects in tertiles of both $A\beta_{1-40}$ and $A\beta_{1-42}$ in the same way as previously described in the Rotterdam Study.¹⁰ However, we were not able to detect a significant increased risk in individuals with the highest tertile of $A\beta_{1-40}$ combined with concentrations in the lowest tertile of $A\beta_{1-42}$ compared with individuals with concentrations in the lowest tertiles of both $A\beta_{1-40}$ and $A\beta_{1-42}$ (figure e-2).

Of the 233 patients with incident dementia, 154 were diagnosed with AD, 46 with mixed or pure vascular dementia, and 33 with dementia due to other causes. A high $A\beta_{1-42}/A\beta_{1-40}$ ratio was significantly associated with a decreased risk of both subtypes of dementia (i.e., AD and mixed/vascular dementia) (table 4). Individuals in the upper $A\beta_{1-42}/A\beta_{1-40}$ tertile had a 2-fold lower risk of developing AD and a 2.9-fold lower risk of developing mixed/vascular dementia (table 4). In contrast, a high $A\beta_{n-42}/A\beta_{n-40}$ ratio was only associated with a lower risk of mixed/vascular dementia (table 4). Individuals in the upper $A\beta_{n-42}/A\beta_{n-40}$ tertile had a nonsignificant 1.4-fold lower risk of AD and a significant 3.7-fold lower risk of mixed/vascular dementia (table 4). Importantly and in accord with our previous results, these associations seemed to be restricted to individuals diagnosed at 2 years of follow-up (table e-4); individuals in the upper $A\beta_{n-42}/A\beta_{n-40}$ tertile had a 5.9-fold lower risk of mixed/vascular dementia (hazard ratio [HR] 0.17, 95% confidence interval [CI] 0.04–0.79, $p = 0.02$; table e-4). Similarly, a high $A\beta_{n-42}/A\beta_{n-40}$ ratio trended to be associated with a lower risk of AD (table e-4). In this case, individuals in the upper $A\beta_{n-42}/A\beta_{n-40}$ tertile had a 1.7-fold lower risk of AD (HR 0.60, 95% CI 0.31–1.06, $p = 0.07$; table e-4).

Last, no association was found between the $A\beta_{1-42}/A\beta_{1-40}$ or $A\beta_{n-42}/A\beta_{n-40}$ ratio on one hand and dementia due to other causes on the other (data not shown). All the associations of the $A\beta_{1-42}/A\beta_{1-40}$ or $A\beta_{n-42}/A\beta_{n-40}$ ratio with the risk of dementia seemed to be independent of the presence or absence of the APOE ε4 allele (data not shown).

DISCUSSION Here, we have shown that an increased $A\beta_{1-42}/A\beta_{1-40}$ plasma ratio is strongly asso-

Table 3 Associations between plasma A β levels and dementia: Hazard ratio (95% confidence interval) for dementia at 2- and 4-year follow-up (n = 233)

	Per SD increase	p	1st Tertile	2nd Tertile	3rd Tertile	p*
Aβ_{1-40}						
Model 1	1.14 (1.00-1.30)	0.06	1.00 (ref)	1.09 (0.78-1.55)	1.49 (1.08-2.07)	0.05
Model 2	1.19 (1.04-1.36)	0.01	1.00 (ref)	1.20 (0.85-1.70)	1.61 (1.16-2.24)	0.03
Aβ_{n-40}						
Model 1	1.15 (1.01-1.32)	0.03	1.00 (ref)	0.95 (0.67-1.34)	1.29 (0.92-1.80)	0.12
Model 2	1.18 (1.04-1.35)	0.01	1.00 (ref)	1.08 (0.76-1.55)	1.49 (1.08-2.01)	0.07
Aβ_{1-42}						
Model 1	0.89 (0.78-1.02)	0.09	1.00 (ref)	0.75 (0.54-1.02)	0.75 (0.52-1.03)	0.10
Model 2	0.91 (0.80-1.04)	0.17	1.00 (ref)	0.80 (0.58-1.11)	0.85 (0.61-1.17)	0.36
Aβ_{n-42}						
Model 1	0.90 (0.78-1.03)	0.13	1.00 (ref)	0.78 (0.57-1.08)	0.68 (0.49-0.95)	0.07
Model 2	0.91 (0.79-1.04)	0.17	1.00 (ref)	0.80 (0.58-1.09)	0.72 (0.52-1.00)	0.13
Aβ_{1-42}/Aβ_{1-40}						
Model 1	0.77 (0.66-0.90)	0.0009	1.00 (ref)	0.73 (0.54-0.99)	0.50 (0.36-0.71)	0.0004
Model 2	0.77 (0.66-0.89)	0.0007	1.00 (ref)	0.72 (0.53-0.97)	0.51 (0.36-0.72)	0.0004
Aβ_{n-42}/Aβ_{n-40}						
Model 1	0.79 (0.68-0.93)	0.004	1.00 (ref)	0.94 (0.69-1.29)	0.68 (0.47-0.95)	0.05
Model 2	0.78 (0.67-0.92)	0.002	1.00 (ref)	0.90 (0.65-1.23)	0.64 (0.45-0.90)	0.03

Model 1: adjusted for center, age, gender, and educational level.

Model 2: adjusted for center, age, gender, educational level, high-density lipoprotein cholesterol, body mass index, diabetes, and APOE status.

*p for trend.

ciated with a decreased risk of incident dementia (AD and mixed/vascular dementia). We also analyzed plasma A β_{n-40} and A β_{n-42} levels for the first

time in a large, prospective, population-based study and observed that an increase in the A β_{n-42} /A β_{n-40} ratio was associated with a decrease in the risk of

Table 4 Associations between plasma A β ratios and dementia subtypes at 2- and 4-year follow-up: Hazard ratio (95% confidence interval) for Alzheimer disease (n = 154) and mixed/vascular dementia (n = 46)

	Per SD increase	p	1st Tertile	2nd Tertile	3rd Tertile	p*
Aβ_{1-42}/Aβ_{1-40}						
Alzheimer disease						
Model 1	0.76 (0.62-0.92)	0.006	1.00 (ref)	0.70 (0.48-1.01)	0.48 (0.31-0.72)	0.002
Model 2	0.76 (0.63-0.92)	0.005	1.00 (ref)	0.71 (0.49-1.04)	0.49 (0.32-0.75)	0.004
Mixed/vascular dementia						
Model 1	0.66 (0.47-0.94)	0.02	1.00 (ref)	0.50 (0.25-1.02)	0.37 (0.17-0.82)	0.03
Model 2	0.67 (0.48-0.94)	0.02	1.00 (ref)	0.46 (0.23-0.93)	0.35 (0.15-0.78)	0.01
Aβ_{n-42}/Aβ_{n-40}						
Alzheimer disease						
Model 1	0.83 (0.66-1.00)	0.06	1.00 (ref)	0.96 (0.63-1.48)	0.76 (0.50-1.16)	0.41
Model 2	0.82 (0.68-0.99)	0.04	1.00 (ref)	0.91 (0.61-1.35)	0.73 (0.48-1.11)	0.31
Mixed/vascular dementia						
Model 1	0.59 (0.40-0.87)	0.009	1.00 (ref)	0.71 (0.36-1.39)	0.32 (0.14-0.79)	0.04
Model 2	0.56 (0.39-0.83)	0.005	1.00 (ref)	0.64 (0.32-1.28)	0.28 (0.11-0.66)	0.01

Model 1: adjusted for center, age, gender, and educational level.

Model 2: adjusted for center, age, gender, educational level, high-density lipoprotein cholesterol, body mass index, diabetes, and APOE status.

*p for trend.

dementia in general and mixed/vascular dementia in particular.

Several studies have been already published to assess the relation between plasma $A\beta$ and dementia.⁶⁻¹³ However, comparisons between these different reports are difficult for different reasons: 1) heterogeneous study design with potential heterogeneous ethnicity background, and differences in follow-up duration and in statistical power; 2) potential fluctuation of $A\beta$ concentrations during the presymptomatic dementia period; and 3) technical issues regarding plasma $A\beta$ quantification.

Interestingly, our data from the 3C Study are in agreement with the relationship between $A\beta_{1-42}/A\beta_{1-40}$ and the risk of dementia initially reported in the Rotterdam Study (i.e., an increase in $A\beta_{1-42}/A\beta_{1-40}$ ratio is strongly associated with a decreased risk of incident AD and mixed/vascular dementia).¹⁰ Both studies presented the same methodologic advantages: a prospective design, a homogeneous white population-based setting, and a large number of participants. These results are also in accord with another study showing that subjects with lower plasma $A\beta_{1-42}/A\beta_{1-40}$ ratios were at greater risk of developing AD,⁶ but not with 3 other recent reports.^{7,12,13} However, these works were based on heterogeneous cohorts including from 227 to 1,075 individuals and with variable follow-up duration. This last point may be particularly important, due to fluctuation of $A\beta$ concentrations during the presymptomatic dementia period.

Indeed, our data clearly indicate that the plasma $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-42}/A\beta_{n-40}$ ratios may be associated with the risk of dementia only in individuals diagnosed at 2 years of follow-up. In the Rotterdam Study, such a difference was not reported over a period of 8 years, but no stratification was shown according to years of follow-up.¹⁰ Furthermore, our observation may be coherent with the recent report describing that conversion to AD was accompanied by a decrease in plasma $A\beta_{1-42}/A\beta_{1-40}$.¹¹ Interestingly, with the establishment of MCI criteria,²² recent studies analyzed the relation between $A\beta$ plasma, MCI entity, and risk to convert to dementia.^{7,12,23,24} The 2 largest MCI studies, one cross-sectional²³ and the other longitudinal,²⁴ showed that $A\beta_{1-42}/A\beta_{1-40}$ ratio is strongly associated with conversion to AD and that highly significant reductions in the $A\beta_{1-42}/A\beta_{1-40}$ ratio are observed during conversion to dementia. Together, these data suggest that variations in plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio may be an indicator of short-term risk of dementia.

Finally, one of the main difficulties to compare the different studies assessing the association of plasma $A\beta$ concentration with risk of dementia re-

sults from the use of different technologies to quantify plasma $A\beta$. Most of these studies have used ELISAs developed from different antibodies, and more recently, a commercial kit has been released using an xMAP liquid phase bead-conjugated array technology. It is important to note that because we assayed plasma $A\beta_{1-40}$, $A\beta_{1-42}$, $A\beta_{n-40}$, and $A\beta_{n-42}$ using a different methodology from that in the Rotterdam Study report, this enabled us to circumvent a specific issue regarding the interpretation of data from ELISAs: it is unclear whether the latter can distinguish between monomeric and oligomeric $A\beta$.²⁵ In contrast, the assay performed in the present study uses an xMAP assay to quantify several epitopes and thus different $A\beta$ species. We also used an xMAP assay that employed different antibodies and thus enabled the quantification of truncated $A\beta$ peptides. Our results were similar to those obtained by measuring plasma $A\beta_{1-40}$ and $A\beta_{1-42}$. Last, as described in previous studies,^{7,11} we observed a strong correlation between plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ (data not shown), indicating that the plasma $A\beta$ peptide concentrations are representative of the physiologic processes leading to $A\beta$ peptide production (i.e., amyloid precursor protein metabolism). Taken as a whole, these various observations seem to reduce (but not eliminate) the likelihood of methodologic bias due to either ELISA- or xMAP-based assay technologies. Finally, the high reproducibility of the xMAP technology was recently demonstrated in a multicenter study,²³ and our data further confirm this observation: the $A\beta_{1-42}/A\beta_{1-40}$ ratio observed in our group without dementia was fully in the range of the ones found in control groups of the 2 other studies using the xMAP technology.^{7,23} The use of a commercially available kit is therefore promising because it could provide comparable, well-standardized measurements in future studies.

Considering the $A\beta_{n-42}/A\beta_{n-40}$ ratio, our results suggest that associations of the $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-42}/A\beta_{n-40}$ ratios with risk of AD and mixed/vascular cases may be not equivalent. To the best of our knowledge, only 1 study has already evaluated relationships between truncated $A\beta$ levels and risk of dementia. The authors did not observe an association between truncated $A\beta$ and the risk of progression from MCI to AD.⁷ However, no data are available regarding a potential association between $A\beta_{n-42}/A\beta_{n-40}$ and mixed/vascular dementia. At this stage of our analysis, it is important to note that in the present work, only 46 individuals have developed mixed/vascular dementia. Further studies are thus clearly needed to confirm or refute a potential relationship between $A\beta_{n-42}/A\beta_{n-40}$ and mixed/vascular dementia risk.

The observed association with the $A\beta_{1-42}/A\beta_{1-40}$ and $A\beta_{n-40}/A\beta_{n-42}$ ratios implies that higher baseline plasma $A\beta_{1-40}$ or $A\beta_{n-40}$ concentrations and greater reductions in plasma $A\beta_{1-42}$ or $A\beta_{n-42}$ concentrations are associated with an increased risk of dementia. However, this type of result is difficult to interpret in the light of our knowledge. First, it is not known whether plasma $A\beta$ peptides reflect a dynamic equilibrium between brain, CSF, and plasma compartments.²⁶⁻³⁰ Second, the source of plasma $A\beta$ species is not known, and the $A\beta$ peptides' physiologic functions are still not fully understood. We cannot exclude the possibility that plasma $A\beta$ may have an impact on the physiologic processes in dementia independently of (or through interaction with) brain $A\beta$ deposits.³¹ To address these different issues and clarify the involvement of plasma $A\beta$ in dementia (AD and mixed/vascular dementia), it will be necessary to run independent, prospective studies with long follow-up periods and repeated measurements of $A\beta$. Furthermore, specific studies will have to address the physiologic role of plasma $A\beta$ peptides and see how these functions may interfere with the dementia process. At this level, several possibilities may be relevant, such as antioxidant³² or vasoconstrictive properties.³³

In conclusion, the present observations strongly suggest that determination of plasma $A\beta$ peptide concentrations (and particularly the $A\beta_{1-42}/A\beta_{1-40}$ ratio) may be useful as a marker of short-term risk of incident dementia (AD and mixed/vascular dementia). For this purpose, the xMAP technology and the use of a commercial kit constitute a high reliable tool for measuring plasma $A\beta$ levels.

AUTHOR CONTRIBUTIONS

Statistical analyses were performed by J.C. Lambert and F. Richard.

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