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Plasma Amyloid-β Levels, Cerebral Small Vessel Disease, and Cognition: The Rotterdam Study

Saima Hilal[a,b], Saloua Akoudad[a,b,c], Cornelia M. van Duijn[d], Wiro J. Niessen[e], Marcel M. Verbeek[f], Hugo Vanderstichele[g], Erik Stoops[g], M. Arfan Ikram[a,b,c] and Meike W. Vernooij[a,b,*]
[a]*Department of Radiology and Nuclear Medicine, Erasmus Medical Center, Rotterdam, The Netherlands*
[b]*Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands*
[c]*Department of Neurology, Erasmus Medical Center, Rotterdam, The Netherlands*
[d]*Genetic Epidemiology Unit, Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands*
[e]*Departments of Radiology Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands*
[f]*Department of Neurology and Laboratory Medicine, Donders Institute for Brain, Cognition and Behaviour, Radboud Alzheimer Centre, Radboud University Medical Center, Nijmegen, The Netherlands*
[g]*ADx NeuroSciences, Gent, Belgium*

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Abstract.
Background: Plasma amyloid-β (Aβ) levels are increasingly studied as a potential, accessible marker of cognitive impairment and dementia. The most common plasma Aβ isoforms, i.e., $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ have been linked with risk of Alzheimer's disease. However, it remains under-explored whether plasma Aβ levels including novel $A\beta_{1\text{-}38}$ relate to vascular brain disease and cognition in a preclinical-phase of dementia
Objective: To examine the association of plasma Aβ levels (i.e., $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$) with markers of cerebral small vessel disease (SVD) and cognition in a large population-based setting.
Methods: We analyzed plasma Aβ1 levels in 1201 subjects from two independent cohorts of the Rotterdam Study. Markers of SVD [lacunes, white matter hyperintensity (WMH) volume] were assessed on brain MRI (1.5T). Cognition was assessed by a detailed neuropsychological battery. In each cohort, the association of Aβ levels with SVD and cognition was performed using regression models. Estimates were then pooled across cohorts using inverse variance meta-analysis with fixed effects.
Results: Higher levels of plasma $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}40}$/ $A\beta_{1\text{-}42}$ ratio were associated with increasing lacunar and microbleeds counts. Moreover, higher levels of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}40}$/ $A\beta_{1\text{-}42}$ were significantly associated with larger WMH volumes. With regard to cognition, a higher level of $A\beta_{1\text{-}38}$ $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}40}$/ $A\beta_{1\text{-}42}$ was related to worse performance on cognitive test specifically in memory domain.
Conclusion: Higher plasma levels of Aβ levels are associated with subclinical markers of vascular disease and poorer memory. Plasma Aβ levels thus mark the presence of vascular brain pathology.

Keywords: Cerebral small vessel disease, cognition, magnetic resonance imaging, plasma amyloid-β levels, population-based

*Correspondence to: Meike W. Vernooij, MD, PhD, Erasmus Medical Center, Office 2505, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands. Tel.: +31 10 703 09 44; E-mail: m.vernooij@erasmusmc.nl.

INTRODUCTION

Cerebral small vessel disease (SVD) is increasingly implicated in the pathophysiology of cognitive impairment and dementia in the elderly [1]. The imaging signature of SVD includes lacunes, microbleeds, and white matter hyperintensities (WMH). Although often subclinical, the presence of these lesions is frequently associated with risk of stroke, cognitive decline, dementia, and mortality [2]. Accumulating evidence suggests that lacunes, WMH, and microbleeds are also linked with cerebral amyloid angiopathy (CAA), which involves deposition of amyloid-β (Aβ) proteins (mainly $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ isoforms) in the cerebral vasculature [3]. In addition to amyloid deposition in the brain, the Aβ peptide levels in the plasma of affected individuals also increases early in the long preclinical-phase of cognitive impairment [4]. Given the greater accessibility of plasma samples in a large-scale setting, there is a considerable interest in examining the circulating levels of Aβ isoforms with Alzheimer's disease (AD) risk [3]. It has been suggested that tracking the process of amyloid abnormalities in asymptomatic individuals may help select persons at higher risk of AD, and preventive treatment, if initiated, could potentially halt the disease process [5].

Previous studies have suggested a link between plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels with AD [6, 7] but high inter-assay variability and differences in study designs have led to conflicting results [8, 9]. With respect to CAA, limited data has reported an abnormal cerebrospinal fluid (CSF) $A\beta_{1\text{-}40}$ /$A\beta_{1\text{-}42}$ ratio among CAA individuals compared to AD [10, 11]. Besides $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, it has been suggested that $A\beta_{1\text{-}38}$ (another isoform produced as a result of abnormal Aβ production), might be a potentially useful biomarker, as it is increased in a disease-specific manner in CSF of AD patients [12]. A combination of $A\beta_{1\text{-}38}$ with other Aβ peptides has been shown to be a sensitive and specific compound marker to identify AD patients over a range of non-Alzheimer dementias. Recently, scant data has shown $A\beta_{1\text{-}38}$ deposition in cerebral vessels without extracellular deposition in sporadic AD in mouse models [12]. To date, it remains under-explored whether plasma Aβ levels including novel $A\beta_{1\text{-}38}$ relate to vascular brain disease and cognition in a preclinical-phase. In this study, we used sensitive colorimetric enzyme-linked immunosorbent assay (ELISA) sandwich test kits that are designed to measure the isoforms of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ levels in Ethylenediaminetetraacetic acid (EDTA) plasma. Using this sensitive technique, we examined the association of plasma $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ levels with SVD and cognition in two independent samples of the Rotterdam Study.

METHODS

Study population

The Rotterdam Study (RS) is a population-based prospective cohort study among middle-age and elderly persons living in the Ommoord district in the city of Rotterdam, the Netherlands. The RS consists of three cohorts where participants undergo follow-up every 3-4 years. The first cohort (RS-I) was initiated in 1990 with 7983 participants at baseline (aged 55 years and above). In 2000, the cohort was extended (RS-II) with 3011 participants aged 55 years and over at that time. In 2006, there was a further extension to the cohort (RS-III) with 3932 persons aged ≥45 years. For the current analyses, two subsamples were drawn from RS-I and RS-III, as plasma samples were not available from RS-II. Persons with prevalent dementia were excluded from both subsamples [13].

For the first subsample, persons were randomly selected ($n = 563$) from RS-I to participate in Rotterdam Scan Study (1995–1996) of whom 490 had gradable magnetic resonance imaging (MRI) [14]. Plasma levels of these persons were collected in the year 1998-1999. Individuals with cortical infarcts and insufficient plasma were excluded leaving 459 persons for the analysis. For the second subsample, a nested case-control design was employed embedded within RS-III (2006–2008). Of 6,057 persons, 3,932 participated in the study, of whom MRI scans were available in 2,956 persons (75%). For the current study, we included 2,923 individuals with gradable T2*-weighted gradient-recalled echo (GRE). Cases were defined as persons with microbleeds ($n = 358$) who were matched on age and sex with controls, i.e. persons without microbleeds ($n = 393$). Individuals with cortical infarcts were eventually excluded resulting in a final sample size of 353 for cases and 389 for controls.

The RS was approved by the Medical Ethics Committee according to the Population Study Act Rotterdam Study, and by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants prior to study recruitment.

Plasma assessment

In both subsamples of RS-I and RS-III, blood samples were drawn in EDTA tubes for plasma collection on the same day. After centrifugation (2500 g, +4°C for 20 min), plasma samples were stored at –80°C within 60 min of collection. Plasma levels of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ were quantified by EUROIMMUN Beta-Amyloid $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ Plasma ELISAs, respectively [15]. This quantification method involved a colorimetric-based sandwich immunoassays (96 wells microplate format) with a readout that can be measured in a microplate reader for absorbance using 450 nm filter. The Aβ were captured by a C-terminal monoclonal antibody ADx104 (4H9), ADx103 (2G3), ADx102 (21F12) coated microplate for $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ amyloid isoforms. The pre-diluted sample (1:4) or calibrator (recombinant full length amyloid isoform) and the biotinylated detector monoclonal antibody ADx101 (3D6) were incubated simultaneously for three hours at room temperature. After a subsequent wash step, addition of tracer (streptavidin-peroxidase), followed by substrate incubation and stopping of the reaction, the analyte level in the samples was calculated using a 4 parameter logistic curve fitting of the 8 non-zero calibrator points. For quality control (QC) purposes, QC samples were produced by pooling of EDTA plasma samples from individual participants. After aliquoting, samples were stored at –80°C. The samples were coded QC1, QC2, QC8, QC9, and QC10 and were used over the three plasma amyloid assays. For the test-run monitoring, five QC samples were included. QC in the three assays were ± 2 standard deviation (SD), with the range of concentration values detected from 5.9–18.7 pg/ml for $A\beta_{1\text{-}38}$, 67.6–161.8 pg/ml for $A\beta_{1\text{-}40}$ and 46.6–55.6 pg/ml for $A\beta_{1\text{-}42}$. The average coefficient of variation of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ measurements in QC plasma samples were 11.04%, 5.72%, and 8.7%, respectively.

Brain imaging

In the subsample of RS-I, brain MRI scans were performed on 1.5T MRI System (VISION MR, Siemens AG, Erlangen, Germany) involving T1-weighted ($1.0 \times 1.0 \times 5.0$ mm^3 voxels; repetition time (TR) 700 ms; echo time (TE) 14 ms; flip angle 70°; matrix 192×256), proton-density ($1.0 \times 1.0 \times 5.0$ mm^3; TR 2200 ms; TE 20 ms; flip angle 80°; matrix 192×256), T2-weighted (TR 2200 ms; TE 80 ms; flip angle 80°; matrix 192×256), and a high-resolution inversion-recovery double contrast three dimensional (3D) HASTE ($1.0 \times 1.0 \times 1.25$ mm^3 voxels; TR 2800 ms; inversion time (TI) 4400 ms; flip angle 180°) sequences [14]. In the subsample of RS-III, brain MRI scans were performed on 1.5T scanner (GE Signa Excite; GE Healthcare, Waukesha, WI) with multisequence protocol comprising of 3D T1-weighted, ($1.0 \times 1.0 \times .1.6$ mm^3 voxels; TR 13.8 ms; TE 2.8 ms; TI 400 ms; flip angle 20°; matrix 416×256), 2D proton density weighted ($1.0 \times 1.0 \times 1.6$ mm^3 voxels; TR 12300 ms; TE 17.3 ms; flip angle 90°; matrix 416×256), 2D fluid attenuated inversion recovery (FLAIR) ($1.0 \times 1.0 \times 2.5$ mm^3 voxels; TR 8000 ms; TE 120 ms; TI 2000 ms; flip angle 90–180°, matrix 320×224) and 3D T2*weighted GRE ($1.0 \times 1.0 \times 1.6$ mm^3 voxels; TR 45 ms; TE 31 ms; flip angle 13°; matrix 320×224) images [16].

Markers of cerebral SVD

Lacunes were defined as lesions involving the subcortical regions, 3–15 mm in diameter, with low signal on T1-weighted image and a high signal on T2-weighted image [17]. Additionally, in the RS-III subsample, the FLAIR sequence was used to identify a hyperintense rim with a center following CSF intensity [18]. Total brain volume and WMH volume were quantified in milliliters by automatic segmentation using a conventional k-nearest-neighbor brain tissue classifier technique [19].

Cerebral microbleeds ratings were only available in RS-III subsample and were graded as small, round to ovoid focal areas of hypointense signal intensity with blooming on 3D T2*-weighted GRE images based on a previously validated method [20]. Microbleeds were classified according to their location into strictly lobar microbleeds and deep/infratentorial microbleeds (with or without lobar microbleeds) as described previously [21].

Cognitive assessment

A detailed neuropsychological test battery was used to assess cognitive function in the two subsamples including Letter Digit Substitution Task, Word Fluency Test, Stroop test (consisting of reading, color-naming, and interference subtasks), and 15-word Verbal Learning Test (consisting of immediate, delayed, and recognition) [22].

Assessment of covariates

Data on demographics and medical history were recorded on the same day of cognitive assessment. Blood pressure was measured in two readings using a random-zero sphygmomanometer in a sitting position, and the mean of both measurements was calculated. Mean arterial blood pressure was calculated as two-thirds of the diastolic blood pressure plus one-third of the systolic blood pressure. Serum total cholesterol levels were measured using an automated enzymatic procedure. Diabetes mellitus was defined as fasting blood glucose $\geq$7 mmol/l, or receiving treatment for diabetes. Smoking was categorized into ever versus never smokers. Education was treated as the number of years of formal education. Distribution of apolipoprotein E (ApoE) genotype and allele frequencies were in the Hardy-Weinberg equilibrium. ApoE ε4 carrier status was defined by the presence of at least one e4 allele. Information on ischemic heart disease was collected during interview which was verified by medical records and was defined as a history of angina or myocardial infarction [23]. Antihypertensive medications included the use of diuretics, β-blocking agents, calcium blockers, angiotensin receptor blockers, and angiotensin-converting enzyme-inhibitors if prescribed for the indication of hypertension.

Statistical analysis within each cohort

To examine differences in the risk factors among participants with presence and absence of microbleeds in RS-III, student T-test was used for normally distributed continuous variable, Mann-Whitney U test for skewed distributed continuous variable, and chi-square test for categorical variables. In both subsamples, all plasma Aβ levels were standardized (by subtracting each variable by population mean divided by SD). The $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio was calculated using these standardized values. For WMH volume, a logarithmical transformation was used to ensure a normal distribution for regression analysis. We first performed Poisson regression models separately in the two subsamples, to determine the association between Aβ levels and lacunes as count data and computed rate ratios (RR) with 95% Confidence Interval (CI). For the Poisson regression model, interpretation of the effect sizes was as follows: a person with one SD increment in Aβ levels will have on average RR times as many lacunes on MRI compared to a person with lower Aβ levels. The similar regression analysis was also conducted to determine the effects of Aβ levels with microbleeds count in the RS-III subsample.

With regards to the continuous variables, linear regression models were used with WMH volume and cognitive test scores as the possible outcomes in each subsample. All the regression models were initially adjusted for age, sex, and subsequently for other covariates which included education (only for cognition), mean arterial blood pressure, total cholesterol, diabetes, smoking, Apoe4 carrier status, ischemic heart disease, and antihypertensive medications. Lastly, in order to determine whether Aβ levels were independently linked with cognition, we additionally adjusted the models for MRI markers of SVD. Statistical analysis was performed using standard statistical software (Statistical Package for Social Science, SPSS V23, SPSS Inc., USA).

Meta-analysis across cohorts

We conducted a meta-analysis of the results obtained from the fully adjusted models in the previous step from the two subsamples using inverse-variance weighting with fixed effects. The analysis of Aβ levels with microbleeds was not included in the meta-analysis due to the absence of these data in the RS-I subsample. Statistical significance was set at p-value <0.05. Correction for multiple comparisons (among 8 cognitive tests) was done using Sidak method, taking into account correlations between variables, with a significance level set at $p \sim 0.008$. Meta-analysis was conducted in Review-Manager 5.3.

RESULTS

Table 1 presents the baseline characteristics of the participants from the two subsamples. In the first-subsample, the mean age of the participants was 67.8 years, and 232 (50.7%) were women. Almost 35.8% used antihypertensive medication whereas the frequency of current smoking was 69.7%. ApoE ε4 carriers were identified in 30.2%, which was comparable with the overall prevalence of ApoE ε4 carriers in the entire Rotterdam Study (28.4%). A total of 100 out of 459 (22.2%) had lacunes whereas the median WMH volume was 8.1 ($\pm$14.5) ml. In the second-subsample consisting of 353 persons

Table 1
Baseline characteristics of the study population

Variables	Subsample of RS-I ($n=459$) 1995–1996	Subsample of RS-III ($n=742$) 2006–2008		p value*
		Cerebral microbleeds		
		Presence ($n=353$)	Absence ($n=389$)	
Demographic and vascular risk factors				
Age (y), mean (SD)	67.8 (7.7)	58.9 (6.98)	58.7 (6.90)	0.743
Women, no. (%)	232 (50.7)	191 (54.1)	210 (54)	0.973
Education (y), mean (SD)	10.8 (3.4)	12.7 (4.09)	13.2 (4.18)	0.100
Mean arterial blood pressure, mmHg, mean (SD)	96.5 (12.7)	100.8 (13.4)	98.7 (12.2)	**0.018**
Antihypertensives, no. (%)	164 (35.8)	98 (28.2)	83 (21.5)	**0.037**
Total cholesterol, mmol/l, mean (SD)	5.7 (0.9)	5.58 (1.12)	5.62 (1.00)	0.544
Antihyperlipidemics, no. (%)	41 (8.9)	94 (27)	92 (23.8)	0.323
Diabetes mellitus, no. (%)	55 (12)	37 (10.5)	35 (9)	0.502
Smoking, ever, no. (%)	318 (69.7)	254 (72)	266 (68.4)	0.288
ApoE ε4 carriers, no. (%)	138 (30.2)	110 (34)	111 (30.7)	0.371
Ischemic heart disease, no. (%)	29 (6.5)	30 (8.5)	9 (2.3)	<0.001
Plasma levels of Aβ isoforms†				
$A\beta_{1\text{-}38}$, pg/ml, mean (SD)	19.4 (4.3)	17.1 (4.16)	16.9 (3.87)	0.568
$A\beta_{1\text{-}40}$, pg/ml, mean (SD)	186.1 (35.9)	159.2 (35.7)	156.1 (32.6)	0.213
$A\beta_{1\text{-}42}$, pg/ml, mean (SD)	56.3 (6.2)	54.9 (6.04)	54.6 (6.43)	0.405
MRI markers				
Presence of lacunes, no. (%)	100 (22.2)	30 (8.5)	17 (4.4)	**0.021**
White matter hyperintensities, ml, median (IQR)	8.1 (14.5)	2.7 (3.51)	2.23 (2.55)	**0.003**
Cognition				
MMSE	27.9 (1.9)	27.9 (1.7)	28 (1.7)	0.052
Letter digit substitution test, mean (SD)	26.8 (7.4)	31.5 (6.5)	31.9 (6.7)	0.307
Stroop test 1, s, mean (SD)	18.3 (4.4)	16.5 (3.5)	16.4 (3.2)	0.618
Stroop test 2, s, mean (SD)	25.5 (6.5)	22.7 (4.4)	22.6 (4.3)	0.575
Stroop test 3, s, mean (SD)	61.9 (29.1)	45.5 (13.8)	44.8 (14.8)	0.557
Word fluency test, mean (SD)	20.5 (5.7)	23.3 (5.8)	23.6 (5.8)	0.493
Immediate recall, mean (SD)	6.7 (1.8)	23.7 (6.5)	24.5 (6.3)	0.089
Delayed recall, mean (SD)	5.9 (2.6)	7.7 (3.1)	8.3 (2.7)	0.007
Recognition, mean (SD)	13.3 (1.9)	13.4 (1.9)	13.6 (1.7)	0.168

RS, Rotterdam Study; SD, standard deviation; No., number; mmHg, millimeters of mercury; mmol/l, millimoles per liter; Aβ, amyloid beta; pg/ml, picogram per milliliters; ml, milliliters; IQR, interquartile range; MRI, magnetic resonance imaging; MMSE, mini mental status examination. *p value denotes differences between presence and absence of cerebral microbleeds. †Plasma samples were collected in the year 1998–1999 for RS-I.

with microbleeds, 253 (71.6%) had a single CMB, 76 (21.5%) had 2–4, and 24 (6.8%) had ≥5 microbleeds. Strictly lobar microbleeds were present in 262 (74.2%), whereas deep/infratentorial microbleeds (with and without lobar microbleeds) in 91 (25.8%) and 61 (17.3%) persons. Individuals with microbleeds had higher mean arterial blood pressure, more often used antihypertensives, had higher burden of ischemic heart disease, and had more lacunes and larger WMH volume compared to those without microbleeds (Table 1). The correlation between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ was (Pearson correlation coefficient, R) = 0.81, between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}42}$ was R = 0.24, and between $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ was R = 0.25.

Meta-analysis of the two subsamples showed that higher levels of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were associated with increasing lacunar counts. Moreover, higher levels of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio were linked with larger WMH volume (Table 2). With respect to microbleeds, higher levels of all Aβ levels including $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio, were significantly associated with increasing microbleeds counts on the scan independent of other risk factors and MRI markers ($A\beta_{1\text{-}38}$ RR = 1.37; 95% CI, 1.26–1.48), ($A\beta_{1\text{-}40}$ RR = 1.48; 95% CI, 1.37–1.62) ($A\beta_{1\text{-}42}$ RR = 1.26; 95% CI, 1.18–1.34) and ($A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ RR = 1.37; 95% CI, 1.21–1.56). Region-specific analysis showed that this association was mainly driven by deep/infratentorial microbleeds (Table 3). As high correlation existed between $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$, we also added these two Aβ isoforms together in the model. The association between $A\beta_{1\text{-}38}$ and lacunar counts remained (RR = 1.23; 95% CI, 1.00–1.51),

whereas it disappeared for $A\beta_{1\text{-}40}$. In contrast, $A\beta_{1\text{-}40}$ was consistently associated with microbleed counts, specifically in the deep/infratentorial region (data not shown).

In terms of cognition, higher $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ levels and $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio were independently linked with delayed recall test [mean difference in scores (per SD increase in $A\beta_{1\text{-}38}$ levels), –0.25; 95% CI, –0.42 to –0.08; $p = 0.003$], [mean difference in scores (per SD increase in $A\beta_{1\text{-}40}$ levels), –0.16; 95% CI, –0.34 to 0.01; $p = 0.06$] and [mean difference in scores (per SD increase in $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio), –0.33; 95% CI, –0.59 to –0.08; $p = 0.01$] in age, sex, education, and cardiovascular risk factor adjusted models. A similar association was also observed with recognition test [mean difference in scores (per SD increase in $A\beta_{1\text{-}38}$ levels), –0.24; 95% CI, –0.36 to –0.12; $p \leq 0.001$], [mean difference in scores (per SD increase in $A\beta_{1\text{-}40}$ levels), –0.21; 95% CI, –0.33 to –0.09; $p \leq 0.001$] and [mean difference in scores (per SD increase in $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio), –0.30; 95% CI, –0.48 to –0.13; $p \leq 0.001$]. After additional adjustment for MRI markers of SVD, these associations remain unaltered. Lastly on applying Sidak correction, the association between $A\beta_{1\text{-}40}$ levels and delayed recall test did not reach this revised level of significance (Table 4).

Analysis of Aβ levels with lacunes, WMH, and cognition within two subsamples are shown in supplementary files. Briefly, a consistent association was observed between higher levels of $A\beta_{1\text{-}38}$ $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio with increasing lacunar counts and worse performance on recognition tests in both subsamples (Supplementary Tables 1 and 2).

DISCUSSION

In this population-based study, higher plasma levels of Aβ, in particular $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio were associated with increasing lacunar counts and larger WMH volume. Furthermore, higher levels of these Aβ were also related to worse cognitive performance, specifically in the memory domain. These findings suggest that plasma amyloid levels may be potential biomarkers for cerebral SVD and cognitive dysfunction in a subclinical phase. Previous studies on plasma Aβ levels and AD risk have reported conflicting results, with some reporting that plasma $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels were raised in AD [6, 7] whereas others have reported reduced levels in mild cognitive impairment and AD patients [4, 24–26]. Conversely, some reports have suggested no differences in plasma $A\beta_{1\text{-}42}$ levels between AD and controls [9, 27, 28]. Within the limits of what circulating plasma Aβ levels may reflect, there is a strong positive correlation between plasma Aβ and Aβ in CSF [29]. In non-demented individuals, CSF and plasma Aβ are in dynamic equilibrium. An increased efflux from CSF to plasma may result in higher plasma concentrations. Prior study on transgenic mouse model of AD has shown significant decreases in CSF and plasma $A\beta_{1\text{-}42}$ levels paralleled with higher $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ concentrations which increases the likelihood of Aβ deposition in the mouse brain tissue [29]. Since there is evidence that plasma concentrations of Aβ change during the preclinical phase, differences in timing of the Aβ measurements with respect to brain amyloid deposition might explain the conflicting results among studies [8]. Indeed, the relationship of plasma Aβ levels and brain amyloid deposition is suggested to be further complicated by dynamics of blood-brain barrier and other possible sources of Aβ materials outside of central nervous system which includes platelets and skeletal muscle cells [30]. However, such a relationship still requires clarification.

With respect to SVD, it has been shown that higher plasma levels of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were associated with severity of WMH and presence of lacunes not only in mild cognitive impairment and AD subjects [4, 9, 31] but also among non-demented elderly participants [32]. However, the latter study was unable to find association of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio with lacunes and WMH. In terms of $A\beta_{1\text{-}38}$, a study on mouse model has demonstrated a predominant localization of $A\beta_{1\text{-}38}$ in the cerebral vasculature of sporadic and familial AD [12]. In addition, $A\beta_{1\text{-}38}$ isoform was also reported to be deposited in the form of extracellular plaques in transgenic AD mouse models [12]. However, until today no data existed in detecting $A\beta_{1\text{-}38}$ levels in human plasma and whether it is an important fluid biomarker for cerebral SVD and cognitive dysfunction. Our findings add further to the previous reports by showing an association of elevated $A\beta_{1\text{-}38}$ levels with lacunes in addition to $A\beta_{1\text{-}40}$ levels. This association remained after adding both $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ into the models. Additionally, we also reported an association of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio with WMH. This may be explained by different mechanisms. First, fibrillary deposition of Aβ in the vessel wall may cause obliteration of the vessel

Table 2
Association of plasma amyloid beta levels with cerebral small vessel disease

Aβ levels (per SD increase)	Lacunar counts RR (95% CI)*	White matter hyperintensities volume Mean difference (95% CI)*
Plasma $A\beta_{1\text{-}38}$ (pg/ml)	**1.30 (1.14–1.48)**	0.04 (–0.01; 0.08), $p = 0.09$
Plasma $A\beta_{1\text{-}40}$ (pg/ml)	**1.30 (1.14–1.48)**	**0.04 (–0.00; 0.09), $p = 0.05$**
Plasma $A\beta_{1\text{-}42}$ (pg/ml)	**1.18 (1.04–1.33)**	–0.00 (–0.04; 0.04), $p = 0.91$
Plasma $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio	1.16 (0.95–1.42)	**0.07 (0.00; 0.14), $p = 0.04$**

Aβ, amyloid beta; SD, standard deviation; pg/ml, picogram per milliliters; RR, rate ratios; CI, confidence interval. *Adjusted for age, sex, mean arterial blood pressure, total cholesterol, diabetes, apolipoprotein ε4 carrier, ischemic heart disease, antihypertensives, and smoking.

Table 3
Association between plasma Aβ1 levels and cerebral microbleeds in the second subsample of RS-II

Aβ levels (per SD increase)	Cerebral microbleeds (counts)			
	All CMBs ($n = 353$) RR (95% CI)*	Strictly lobar CMBs ($n = 262$) RR (95% CI)*	Deep/infratentorial CMBs† ($n = 91$) RR (95% CI)*	Strictly deep/ infratentorial CMBs ($n = 61$) RR (95% CI)*
Plasma $A\beta_{1\text{-}38}$ (pg/ml)	**1.37 (1.26–1.48)**	1.04 (0.92–1.16)	**1.96 (1.65–2.34)**	**1.39 (1.07–1.83)**
Plasma $A\beta_{1\text{-}40}$ (pg/ml)	**1.48 (1.37–1.62)**	1.11 (0.98–1.26)	**2.07 (1.78–2.39)**	**1.71 (1.26–2.30)**
Plasma $A\beta_{1\text{-}42}$ (pg/ml)	**1.26 (1.18–1.34)**	0.92 (0.83–1.02)	**1.47 (1.34–1.63)**	1.16 (0.95–1.41)
Plasma $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio	**1.37 (1.21–1.56)**	1.23 (0.99–1.47)	**1.89 (1.46–2.45)**	**1.63 (1.08–2.46)**

Aβ, amyloid-β; SD, standard deviation; pg/ml, picogram per milliliters; CMB, cerebral microbleeds; RR, rate ratios; CI, confidence interval. *Adjusted for age, sex, mean arterial blood pressure, total cholesterol, diabetes, apolipoprotein ε4 carrier, ischemic heart disease, antihypertensives, and smoking. †With lobar CMB.

lumina, loss of vascular smooth muscle tone, and endothelial damage leading to hypoperfusion and cerebrovascular damage [32]. Second, overproduction of Aβ may lead to deposition in the media and adventitia of cortical arterioles and arteries which may be further supported by Apoe4 isoform which facilitates transformation of β sheet into Aβ oligomers [33]. Third, soluble Aβ may directly affect cerebral vasoreactivity by enhancing endothelin-1 induced vasoconstriction. This altered autoregulation may affect brain regions which limited collateral circulation resulting in lacunar infracts and white matter hyperintensities [34]. Fourth, reduced cerebral blood flow or hypoperfusion upregulates amyloid-β protein precursor expression and thereby promotes production of Aβ in endothelial cells leading to its secretion in the peripheral circulation [35].

In addition to lacunes and WMH, we reported an association of Aβ levels with cerebral microbleeds in the RS-III subsample. Studies comparing patients with CAA and AD have shown lower levels of CSF $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in CAA pathology [10, 11]. However, data examining the plasma Aβ levels in CAA pathology are scarce with controversial results. One study has shown higher levels of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in plasma of patients with CAA-related intracranial hemorrhage [36] whereas the other study did not show alterations in the plasma levels of Aβ in CAA induced hemorrhage compared to controls [37]. In this study, we specifically studied plasma Aβ levels in a sub-sample of the general population enriched with microbleeds, as our previous studies have consistently shown that lobar microbleeds may reflect subclinical CAA. Contrary to what we expected, we found that higher levels of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40,}$ and $A\beta_{1\text{-}42}$ were primarily associated with deep and infratentorial microbleeds and not with lobar microbleeds (though the effect estimates were in the direction of increased risk albeit non-significant). The possible reason for this discrepancy could be that plasma Aβ levels may reflect vascular disease in the brain other than vascular amyloid deposition. Previously, deep and infratentorial microbleeds have been linked to hypertensive vascular pathology and are presumed not to be accompanied by neuronal degeneration secondary to amyloid deposition in the brain [38]. We postulate that the vasoactive properties of Aβ in combination with high blood pressure can upregulate angiotensin converting enzyme (ACE) and subsequently induce adverse changes such as inflammation, imbalance of oxygen free radicals, and apoptosis [39] contributing to development of microbleeds.

With regards to cognition, limited data has shown that a higher $A\beta_{1\text{-}40}$ level was associated with poorer

Table 4
Association of plasma amyloid-β levels with cognition

Aβ levels (per SD increase)	LDST β (95% CI)*, *p* value	Stroop test 1 β (95% CI)*, *p* value	Stroop test 2 β (95% CI)*, *p* value	Stroop test 3 β (95% CI)*, *p* value	Word fluency test β (95% CI)*, *p* value	Immediate recall β (95% CI)*, *p* value	Delayed recall β (95% CI)*, *p* value	Recognition β (95% CI)*, *p* value
Plasma $A\beta_{1-38}$	–0.08 (–0.48; 0.33)	–0.13 (–0.34; 0.08)	0.09 (–0.21; 0.39)	0.21 (–0.83; 1.26)	–0.12 (–0.48; 0.24)	**–0.14 (–0.25; –0.02)**	**–0.26 (–0.43; –0.09)**	**–0.24 (–0.35; –0.12)**
	p = 0.71	*p* = 0.22	*p* = 0.67	*p* = 0.69	*p* = 0.52	***p* = 0.02**	***p* = 0.003**†	***p* ≤ 0.001**†
Plasma $A\beta_{1-40}$	–0.02 (–0.40; 0.44)	**–0.22 (–0.44; –0.00)**	–0.03 (–0.35; 0.28)	0.09 (–0.98; 1.17)	–0.14 (–0.50; 0.23)	–0.08 (–0.20; 0.04)	**–0.18 (–0.36; –0.00)**	**–0.21 (–0.33; –0.09)**
	p = 0.92	***p* = 0.05**	*p* = 0.83	*p* = 0.86	*p* = 0.47	*p* = 0.19	***p* = 0.05**	***p* ≤ 0.001**†
Plasma $A\beta_{1-42}$	0.08 (–0.30; 0.46)	–0.05 (–0.25; 0.15)	–0.05 (–0.33; 0.24)	–0.00 (–0.97; 0.97)	–0.28 (–0.62; 0.05)	–0.03 (–0.14; 0.08)	0.09 (–0.07; 0.25)	–0.02 (–0.13; 0.09)
	p = 0.67	*p* = 0.61	*p* = 0.75	*p* = 0.99	*p* = 0.10	*p* = 0.59	*p* = 0.28	*p* = 0.74
Plasma $A\beta_{1-40}$/ $A\beta_{1-42}$ ratio	–0.02 (–0.63; 0.06)	–0.14 (–0.46; 0.18)	0.08 (–0.37; 0.54)	0.49 (–1.07; 2.04)	0.15 (–0.40; 0.70)	–0.10 (–0.28; 0.09)	**–0.37 (–0.63; –0.10)**	**–0.28 (–0.46; –0.10)**
	p = 0.96	*p* = 0.38	*p* = 0.72	*p* = 0.54	*p* = 0.59	*p* = 0.30	***p* ≤ 0.001**†	***p* = 0.002**†

Aβ, amyloid-β; SD, standard deviation; pg/ml, picogram per milliliters; LDST, letter digit substitution test; β, mean difference; CI, confidence interval. *Adjusted for age, sex, education, mean arterial blood pressure, total cholesterol, diabetes, apolipoprotein ε4 carrier, smoking, ischemic heart disease, antihypertensives, and presence of other SVD marker. †Statistically significant after Sidak correction (~0.008).

memory and information processing speed, as well as cognitive decline in cognitively normal adults [40]. However, no study has examined $A\beta_{1-38}$ levels in relation to cognitive performance and whether individuals show different patterns of impairment in cognitive domains. Our study showed that higher levels of both $A\beta_{1-38}$ and $A\beta_{1-40}$ and $A\beta_{1-40}/A\beta_{1-42}$ ratio in asymptomatic older adults were associated with worse cognition in delayed and recognition word learning tests, which reflect memory domains. No associations were observed between $A\beta_{1-42}$ and cognitive functioning. It has been suggested that higher plasma Aβ levels possibly trigger cortical thinning and reduced grey matter density in temporal lobes, disrupting important cognitive networks leading to cognitive dysfunction [41, 42]. Strong correlations have been reported previously between CSF $A\beta_{1-38}$ and $A\beta_{1-40}$ levels as they are thought to originate from the same product line (tripeptide release) by gamma-secretase [12] whereas $A\beta_{1-42}$ is reported to originate independent of $A\beta_{1-38}$ [42,43]. Moreover, it has also been demonstrated that both $A\beta_{1-38}$ and $A\beta_{1-40}$ isoforms exhibit similar spatial distribution in a number of blood vessels, due to higher solubility and sparse aggregation of these isoforms, which may explain their higher levels in plasma compared to other Aβ peptides [12]. Therefore, higher $A\beta_{1-38}$ and $A\beta_{1-40}$ levels in asymptomatic older adults might be sensitive markers of accelerated aging and may reflect vascular damage in subcortical regions as revealed by their link with impaired memory domain.

Strengths of our study include: population-based design, use of extensive neuropsychological battery to assess cognitive function and use of a colorimetric ELISA technique to measure $A\beta_{1-38}$, $A\beta_{1-40}$, and $A\beta_{1-42}$ levels. There are several potential limitations to consider. First, there was a 3-year time window between plasma collection and MRI assessment in the first subsample of RS-I which may over- or underestimate effect sizes. However, this limitation was overcome by performing a similar analysis in the younger cohort, in whom MRI and plasma measurement were performed at the same time. Our results consistently showed that plasma Aβ levels were associated with SVD and cognition within studies and in meta-analysis. Second, RS-III subsample was enriched with participants with cerebral microbleeds, which limits the generalizability of the results to a general population. Third, as the data was examined cross-sectionally, the temporal relationship between Aβ levels, SVD, and cognition cannot be established. Fourth, the microbleed and lacunar counts used in

the present analysis may not reflect the true biological number because the detection of microbleed and lacunes on MRI strongly depends on technical imaging methods used (e.g., field strength and spatial resolution). Finally, microbleeds as detected on MRI may provide an indirect measure of CAA or amyloid deposition and other imaging modalities such as amyloid positron emission tomography could allow more accurate measurement of total (vascular and parenchymal) amyloid deposition in the brain.

In conclusion, our results suggest that high plasma Aβ levels, in particular $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$, in non-demented subjects are associated with subclinical markers of vascular brain disease and with worse performance on cognitive tests specifically memory. Future studies should examine whether inclusion of novel plasma $A\beta_{1\text{-}38}$ levels as an additional biomarker can provide further information on microvascular damage in the brain. This observation might be useful for future experiments involving use of gamma-secretase modulators in preclinicalAD.

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SUPPLEMENTARY MATERIAL

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