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Plasma amyloid-β40 in relation to subclinical atherosclerosis and cardiovascular disease: A population-based study

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ABSTRACT

Background and aims: We aimed to determine associations of plasma amyloid-β40 (Aβ40) with subclinical atherosclerosis and risk of atherosclerotic cardiovascular disease (ASCVD) in the general population.
Methods: Between 2002 and 2005, plasma Aβ40 was measured by single molecule array (SiMoA®) in 3879 participants of the population-based Rotterdam Study (mean age: 71 years, 61% female). Subclinical atherosclerosis was quantified as computed tomography-assessed calcification volumes. We determined the association of Aβ40 with calcification volumes and clinical ASCVD event risk, and repeated the analyses for ASCVD in a replication cohort of 1467 individuals.
Results: Higher levels of Aβ40 were associated with increased volumes of calcification in the coronary arteries and to a lesser extent extracranial carotid arteries, independent of traditional cardiovascular risk factors. Of all 3879 participants, 748 developed ASCVD during a median 9.7 years of follow-up. In age- and sex-adjusted models, higher Aβ40 predisposed to a minor increase in ASCVD risk (HR [95%CI]: 1.11[1.02–1.21] per 1-SD increase in Aβ40), driven by coronary heart disease (HR: 1.17[1.05–1.29]) rather than stroke (HR: 1.04 [0.93–1.16]). However, excess risk of clinical outcomes was largely explained by baseline differences in cardiovascular risk factors and attenuated after further adjustment (for ASCVD– HR: 1.05[0.96–1.15] and for CHD– HR: 1.08[0.96–1.20]). Results were similar in the replication cohort, with highest risk estimates for CHD (HR: 1.24[1.04–1.48]) in age- and sex-adjusted models, attenuated after adjustment for cardiovascular risk factors (HR: 1.15[0.96–1.39]).
Conclusions: In this population-based study, higher plasma amyloid-β40 is associated with subclinical atherosclerosis, but not risk of first-ever ASCVD after accounting for traditional cardiovascular risk factors.

1. Introduction

Amyloidosis is characterised by the aggregation of abnormal proteins in a wide range of tissues and organs systems. Among the various types of amyloid fibrils, amyloid-β is the hallmark of Alzheimer's disease in the brain. Consequently, amyloid-β has been studied chiefly in the context of dementia, even though it is produced outside of the central nervous system in multiple cell types including blood platelets and endothelial cells [1–3]. Amyloid-β monomers are derived here in varying length through cleavage of the amyloid precursor protein. The 42-residue long monomer (Aβ42) is most common in cerebral depositions with Alzheimer's disease, whereas amyloid-β40 (Aβ40) is

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abundant in the circulation and contributes to stroke and vascular cognitive impairment through cerebral amyloid angiopathy [4,5]. Vascular effects of Aβ40, however, may extend well beyond the cerebral vasculature, potentially explaining the widely observed link between cardiovascular and neurodegenerative disease [5–8].

Recent clinical studies have indeed found that plasma levels of Aβ40 are elevated in patients with coronary heart disease (CHD) [9–11], and that higher Aβ40 levels are associated with increased risk of cardiovascular mortality in patients with stable coronary artery disease or myocardial infarction [12,13]. The underlying mechanisms remain uncertain, but may relate to atherosclerotic burden [12], hypertension inducing effects of Aβ40 [1,14–16], and vascular inflammation [17–19]. The incremental value of Aβ40 for predicting prognosis in patients with CHD [13], further suggests that Aβ40 could be a marker for atherosclerotic cardiovascular disease (ASCVD) event risk in the general population. Yet, the association of Aβ40 with first-ever manifestations of ASCVD is undetermined.

We determined the association of plasma Aβ40 levels with risk of ASCVD, including CHD and stroke, in a population-based setting, and explored pathophysiological substrates by investigating Aβ40 in relation to subclinical atherosclerosis in different vessel beds.

2. Patients and methods

This study is embedded within the Rotterdam Study, a large ongoing population-based cohort study in the Netherlands among individuals aged ≥40 years residing in the Ommoord area, a suburb of Rotterdam. The Rotterdam Study methods have been described in detail previously [20]. In brief, participants are interviewed at home and subsequently examined at the research centre every 4 years since inception of the cohort in 1990. For the current study, we used data from the fourth examination of the original cohort (RS-I) and the second examination of the cohort expansion (RS-II) of the Rotterdam Study. Between 2002 and 2005, 5405 participants (72% of invitees) visited the research centre for physical examination, 5094 of whom had bloods samples taken. For replication, we used plasma amyloid-β measurements from 1834 participants, whom –for reasons of efficiency– had previously been selected randomly from all 7157 attendees at the baseline study assessment from 1990 to 1993 [21]. A flow chart of participant inclusion is shown in Supplemental Fig. S1.

2.1. Ethics statement

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. Written informed consent was obtained from all participants.

2.2. Data availability statement

Anonymised data are available on reasonable request. All data relevant to the study are included in the article or uploaded as online supplemental information. Requests for access to the data reported in this paper can be directed to data manager Frank J.A. van Rooij (secretariat.epi@erasmusmc.nl).

2.3. Patient and public involvement statement

Participants of the Rotterdam Study are represented in a panel that provides regular input on study affairs. Results are disseminated to participants by periodic newsletters. No participants were specifically involved in the research question of the current study.

2.4. Plasma amyloid measurement

At centre visit, EDTA plasma was sampled, aliquoted, and frozen at −80 °C according to standard procedures. Samples were not thawed prior to amyloid measurement. Measurements were carried out in two separate batches; the first batch included 2,000 samples, obtained from a random selection of 1,000 participants from the fourth visit of RS-I and 1,000 from the second visit of RS-II. The second batch included samples from all the remaining participants of these two study waves. All measurements were performed at Quanterix (Lexington, MA, USA) on a single molecule array (SiMoA) HD-1 analyzer platform [22]. The SiMoA Human Neurology 3-Plex A assay was used for measuring the concentration of Aβ40. Samples were tested in duplicate, and two quality control samples were run on each plate for each analyte. We excluded samples for which no duplicate was available, for which control samples were out of range, or when the concentration coefficient of variation exceeded 20% [22]. Inter- and intra-assay variation are reported in Supplementary Table S1.

In the replication cohort, non-fasting blood samples were obtained by venepuncture at baseline, and plasma concentrations of Aβ40 were determined by a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) method (Pfizer, USA). Detailed methods have been described previously [21]. Distributions of the Aβ40 for the main cohort and replication cohort are presented in Supplemental Fig. S2.

2.5. Quantification of atherosclerosis

In a random subset of individuals, non-contrast CT images were obtained using 16-slice (n = 657) or 64-slice (n = 1585) multidetector CT scanners (Somatom Sensation 16 or 64; Siemens, Forchheim, Germany). We obtained images of the coronary arteries, extracranial carotid arteries, and intracranial carotid arteries, using a field-of-view optimized for visualization of blood vessels. Imaging parameters have been described previously [23]. Calcification of the epicardial coronary arteries and extracranial carotid artery were volumetrically quantified using commercially available software (Syngo CalciumScoring; Siemens). Extracranial carotid artery calcification was measured within 3 cm proximal and distal of the bifurcation. Intracranial carotid artery calcification was evaluated from the horizontal segment of the petrous internal carotid artery to the bifurcation into the anterior and medial cerebral artery, using a semi-automated scoring method that allows to manually delineate calcification from ossal structures and calculate the volume by multiplying the number of pixels above the threshold for calcification (i.e., 130 Hounsfield units) with the pixel size and slice increment [24].

2.6. Assessment and follow-up for ASCVD

Participants underwent extensive examinations at a dedicated research centre at baseline and every 4 years henceforth, including structured interview about their past medical history, current medication use, and 12-lead resting electrocardiogram (ECG) [20]. The information from in-person screening was supplemented by data from the electronic linkage of the study database with medical records from all general practitioners, in-hospital consultants, and care home physicians. With this linkage, the entire cohort is continuously monitored for verification of diagnosis at centre visits, and detection of interval cases of disease between centre visits. Information on vital status was additionally obtained through a bimonthly check of municipal records. ASCVD was defined as a composite of CHD and ischaemic stroke/TIA.

CHD consisted of the composite endpoint of myocardial infarction, coronary revascularisation procedures (i.e., coronary artery bypass grafting or percutaneous coronary intervention), and coronary mortality [25]. Diagnosis of myocardial infarction and coronary revascularisation

during follow-up is based on information from medical records [25]. For each cardiac outcome, two research physicians independently classified information accordingly, after which a panel headed by a consultant cardiologist adjudicated the final diagnosis [25].

Stroke and TIA were defined according to WHO criteria as a syndrome of rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms leading to death or lasting ≥24 h for stroke and <24 h for TIA, in the absence of an apparent non-vascular cause [26]. Diagnosis of stroke and TIA were based on medical records and (verified) self-reported medical history. Similar to CHD diagnosis, medical records from general practitioners and hospital discharge letters were collected and reviewed by research physicians, after which a consensus panel headed by an experienced vascular neurologist decided on the diagnosis.

All researchers partaking in case ascertainment were blinded to participants' amyloid levels. Follow-up until January 1, 2015 was virtually complete (>96% of potential person years).

2.7. Other measurements

At baseline, we assessed smoking status (i.e. current, former, never), and use of blood pressure lowering, lipid-lowering, and anti-thrombotic medication by interview. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol, haemoglobin levels, C-reactive protein (CRP), and creatinine were measured at baseline. Because CRP and creatinine were measured in a random half of participants only, missing values were complemented with the last observation carried forward from the previous centre visit 4 years prior. Blood pressure was measured with a random-zero sphygmomanometer. Hypertension was defined as a blood pressure >140/90 mmHg or the use of blood-pressure lowering medication. Body mass index (BMI) was computed from measurements of height and weight (kg/m^2). Diabetes was defined as the use of blood glucose-lowering medication or a fasting serum glucose level ≥7.0 mmol/L.

2.8. Analysis

Of all 5094 eligible participants who had blood samples taken, 4853 (95.3%) passed quality control for the Aβ40 measurement. Of these, 3879 participants who were free of ASCVD at baseline were included in the analyses on subclinical atherosclerosis and first-ever ASCVD event risk. Missing covariate data (≤5.5% for all covariates) were imputed using fivefold multiple imputation. Distribution of covariates was similar in the imputed versus the non-imputed dataset.

First, we determined the association of plasma Aβ40 with cardiometabolic risk factors using univariable and multivariable linear regression models, applying the Benjamini-Hochberg method to account for multiple testing of statistical significance.

We then determined the association of Aβ40 with subclinical atherosclerosis on CT in the coronary arteries, extracranial carotid arteries, and intracranial carotid arteries, using analysis of variance (ANOVA). Differences in absolute levels of Aβ40 facilitate comparison with prior studies. Models incorporated scanner type in addition to age, sex, batch number, smoking habits, systolic and diastolic blood pressure, use of blood pressure-lowering medication, serum total and HDL cholesterol, use of lipid-lowering medication, diabetes, BMI, use of antithrombotic medication, haemoglobin, eGFR, and CRP. We stratified analyses by age and sex, as well as hypertensive status and CRP levels, as these have been suggested of importance in the physiological effect of Aβ40 [1]. We formally tested for interaction on the multiplicative scale and additive scale (i.e., relative excess risk due to interaction [RERI]) in a generalised linear model.

Next, we determined the association of plasma Aβ40 with incident ASCVD, and separately for CHD and ischaemic stroke/TIA, using Cox regression models. Participants were censored within the follow-up period at date of ASCVD event, death, loss to follow-up, or administrative censoring date, whichever came first. The proportional hazard assumption was met, and tests for non-linearity were not statistically significant (Supplemental Fig. S3). All analyses were adjusted for age, sex and batch, and additionally in a second model for the same covariates as the CT-imaging analysis. We then again stratified analyses as detailed above, similarly testing for interaction in the Cox model. We repeated analyses after excluding all participants who developed dementia during the study period.

Analyses were repeated among 1467/1834 participants in the replication cohort who had valid measurements of plasma Aβ40 and had no clinical history of ASCVD at baseline. To prevent overlap in follow-up time, 549 of these 1467 individuals who were part of both the main cohort and the replication cohort were thereby censored before start of the main cohort examination round in April 2002.

Analyses were done using IBM SPSS Statistics version 25.0 (IBM Corp, Armonk, NY, USA) and R version 4.0.5 (packages 'survminer' and 'epiR').

Table 1
Baseline characteristics.

	Main study cohort		Replication cohort
	Overall population (N = 3879)	CT-imaging subsample (N = 1938)	(N = 1467)
Age (years)	71.3 (±7.3)	68.5 (±6.5)	68.1 (±8.7)
Female sex	2353 (60.7%)	1051 (54.2%)	935 (63.7%)
Systolic blood pressure (mmHg)	149 (±21)	146 (±20)	138 (±22)
Diastolic blood pressure (mmHg)	80 (±11)	81 (±11)	73 (±11)
Blood pressure lowering medication	1511 (39.2%)	664 (34.7%)	376 (25.6%)
Diabetes mellitus	470 (12.9%)	220 (12.0%)	88 (6.2%)
Body mass index (kg/m^2)	27.6 (±4.1)	27.6 (±3.9)	26.2 (±3.6)
Serum total cholesterol (mg/dL)	222 (±37)	223 (±37)	258 (±48)
Serum high-density lipoprotein (mg/dL)	57 (±15)	56 (±15)	53 (±14)
Lipid-lowering medication	645 (16.7%)	345 (18.0%)	24 (1.6%)
Smoking			
Former	2006 (52.7%)	1016 (53.6%)	574 (39.8%)
Current	587 (15.4%)	300 (15.8%)	316 (21.9%)
Antithrombotic medication	632 (16.4%)	255 (13.3%)	44 (3.0%)
Glomerular filtration rate (mL/min/1.73 m^2)	78 (±15)	80 (±15)	76 (±15)
Haemoglobin (g/dL)	14.1 (±1.2)	14.3 (±1.2)	14.1 (±1.3)
C-reactive protein (mg/L; median, IQR)	1.6 (0.7–3.2)	1.4 (0.5–2.9)	1.7 (0.8–3.5)
Amyloid-β40 (pg/mL; median, IQR)	255 (227–288)	246 (220–276)	190 (162–224)
Amyloid-β40 (pg/mL; mean, SD)	260 (±52)	251 (±47)	197 (±55)

SD = standard deviation; IQR = interquartile range. Data are presented as frequency (%) for categorical, and mean ± SD for continuous variables unless indicated otherwise.

Table 2
Determinants of plasma amyloid-β40.

	Univariable models Mean difference (95% CI)	Multivariable model[a] Mean difference (95% CI)
Age (per decade)	**0.53 (0.49;0.57)*****	**0.36 (0.32;0.40)*****
Male sex	−0.04 (−0.10; 0.03)	**0.11 (0.04;0.18)****
Systolic blood pressure (per 10 mmHg)	**0.05 (0.03;0.06)*****	0.006 (−0.01; 0.02)
Diastolic blood pressure (per 10 mmHg)	**−0.11 (-0.14;-0.08)*****	**−0.05 (-0.08;-0.01)****
Blood pressure lowering medication	**0.40 (0.33;0.46)*****	**0.22 (0.15;0.28)*****
Diabetes mellitus	**0.18 (0.08;0.28)*****	0.09 (−0.001; 0.18)
Body-mass index (kg/m^2)	−0.01 (−0.02; 0.001)	**−0.01 (-0.02;-0.01)*****
Serum cholesterol (per 10 mg/dL)	**−0.02 (-0.03;-0.01)*****	−0.001 (−0.01; 0.01)
Serum high-density lipoprotein (per 10 mg/dL)	**−0.06 (-0.08;-0.04)*****	**−0.05 (-0.07;-0.03)*****
Lipid-lowering medication	**0.10 (0.02;0.18)***	0.04 (−0.04; 0.12)
Smoking (compared with never smokers)		
Former	−0.06 (−0.14; 0.01)	−0.02 (−0.08; 0.04)
Current	−0.08 (−0.18; 0.02)	**0.16 (0.07;0.25)*****
Antithrombotic medication	**0.30 (0.21;0.38)*****	−0.02 (−0.10; 0.06)
Haemoglobin (g/dL)	**−0.14 (-0.16;-0.11)*****	**−0.08 (-0.11;-0.06)*****
Estimated glomerular filtration rate (per 10 mL/min/1.73 m^2)	**−0.03 (-0.03;-0.02)*****	**−0.02 (-0.02;-0.02)*****
C-reactive protein (natural log of mg/L)	**0.12 (0.09;0.15)*****	**0.04 (0.01;0.07)****

*$0.05 < p \geq 0.01$; **$0.01 < p \geq 0.001$; ***$p < 0.001$; results in bold are significant at $\alpha = 0.05$ after accounting for multiple testing.
[a] Includes all variables presented in the table, plus batch number.

3. Results

Baseline characteristics of 3879 participants in the overall cohort, and the subset of 1938 participants without ASCVD who underwent non-enhanced CT are presented in Table 1. Participants with CT-imaging on average were slightly younger with consequent lower levels of Aβ40, but had a similar cardiovascular risk profile as participants in the overall cohort.

3.1. Determinants of plasma Aβ40

In multivariable models, plasma Aβ40 levels were higher with advancing age (β [95%CI] per decade: 0.36 [0.32–0.40]), and higher in men than in women (β = 0.11 [0.04–0.18]; Table 2, and per quartile in Supplemental Table S2). Other characteristics that were significantly associated with higher Aβ40 after correction for multiple testing were use of antihypertensive medication, diastolic blood pressure, current smoking, HDL cholesterol, renal function, CRP, anaemia, and lower BMI (Table 2).

3.2. Aβ40 and subclinical atherosclerosis

Among 1938 participants with CT-imaging who had no clinical history of ASCVD, Aβ40 was associated with volumes of arterial calcification in the coronary arteries (any calcification vs. none: $p = 0.0008$), and to a lesser extent in the extracranial carotid arteries ($P = 0.05$) (Fig. 1). No significant associations were seen with intracranial carotid artery calcification (Fig. 1; numerical data are presented in Supplemental Table S3).

The association of Aβ40 with coronary calcification was more profound in participants with uncontrolled hypertension than in those without hypertension (Fig. 2; $p = 0.046$ for interaction, and RERI [95% CI] = 0.77 [0.05–1.49], indicating positive interaction). We observed no significant effect modification for coronary calcification by age, sex, or CRP (Fig. 2), nor did we find any evidence of effect modification for carotid artery calcification (Supplemental Fig. S4).

3.3. Aβ40 and ASCVD event risk

During 33,342 person years of follow-up (median 9.7 years), 749 individuals developed a first ASCVD event, of whom 399 had CHD and 390 had a stroke or TIA as their first ASCVD event. In the age- and sex-adjusted models, higher Aβ40 was associated with a slight increase in ASCVD risk (HR 1.10 [1.02–1.19] per 1-SD increase in Aβ40), driven by higher risk of CHD (HR 1.17 [1.05–1.29]; Table 3). Risk estimates were attenuated and no longer statistically significant after adjustment for baseline cardiovascular risk factors (for all ASCVD– HR: 1.05 [0.96–1.14]; and for CHD– HR: 1.08 [0.96–1.20]; Table 3). This was due notably to use of antihypertensive medication and serum lipid levels (Supplemental Table S4). Fully adjusted survival curves are presented in Fig. 3.

Similar to the association between Aβ40 and coronary calcification, effect estimates for Aβ40 were somewhat higher in individuals with uncontrolled hypertension (Fig. 2), although formal tests for interaction did not support this statistically ($p = 0.79$; RERI [95% CI] = 0.10 [−0.26; 0.46]). Again, we observed no significant effect modification by age, sex, or CRP (Fig. 2). There was no consistent evidence of effect modification by any of these variables for ischaemic stroke

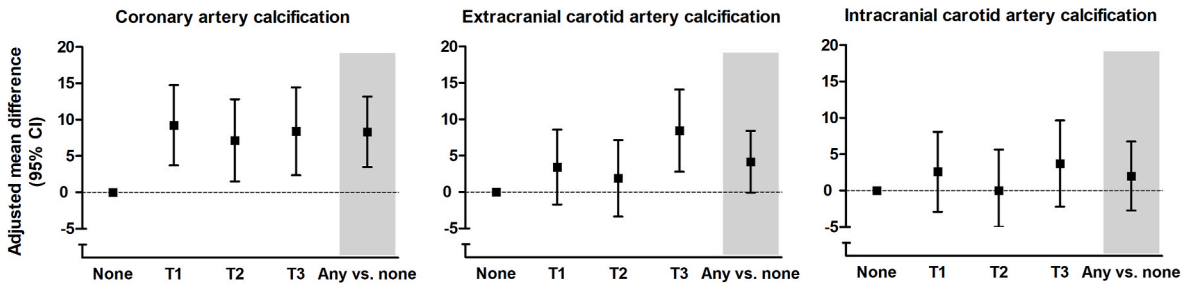

Fig. 1. Amyloid-β40 and subclinical atherosclerosis in different vessel beds.
The figure depicts the absolute mean difference in plasma Aβ40 levels, comparing individuals across tertiles of calcification volume (mm^3) *versus* individuals without calcification. Tertile cut-offs are presented in online Supplemental Table S2.

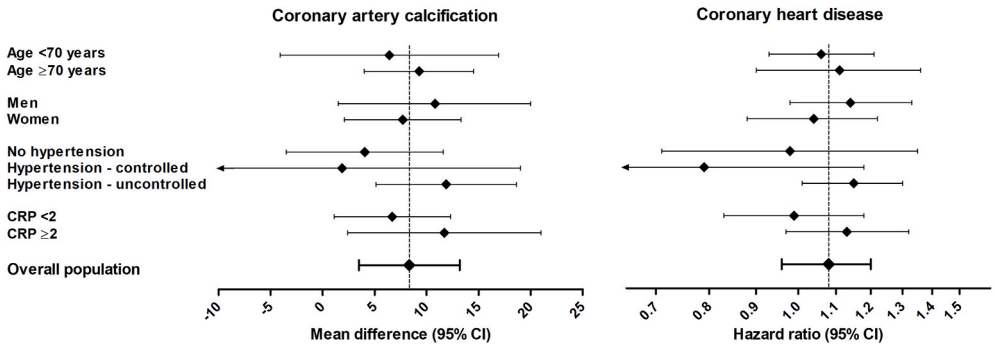

Fig. 2. Association of amyloid-β40 with subclinical atherosclerosis and clinical CHD in various subgroups.
The figure depicts the absolute difference in plasma Aβ40 levels between participants with and without coronary calcification, and the hazard ratio for clinical coronary heart disease per 1 standard deviation increase in Aβ40. Results are from the fully adjusted models.

Table 3
Amyloid-β40 and primary atherosclerotic cardiovascular disease event risk during follow-up.

	Atherosclerotic cardiovascular disease (n/N = 749/3879)	Coronary heart disease (n/N = 399/3879)	Ischaemic stroke/TIA (n/N = 390/3879)
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Model I			
Q1 (<227 pg/mL)	Reference	Reference	Reference
Q2 (227–255 pg/mL)	1.05 (0.85–1.30)	1.08 (0.79–1.46)	1.10 (0.82–1.48)
Q3 (256–287 pg/mL)	1.09 (0.88–1.35)	1.12 (0.83–1.51)	1.10 (0.82–1.48)
Q4 (>287 pg/mL)	1.24 (0.99–1.54)	**1.44 (1.07–1.95)**	1.08 (0.80–1.47)
Per 1-SD increase	**1.10 (1.02–1.19)**	**1.17 (1.05–1.29)**	1.04 (0.93–1.16)
Model II			
Q1 (<227 pg/mL)	Reference	Reference	Reference
Q2 (227–255 pg/mL)	1.07 (0.86–1.34)	1.11 (0.82–1.51)	1.11 (0.83–1.50)
Q3 (256–287 pg/mL)	1.04 (0.83–1.29)	1.05 (0.77–1.42)	1.07 (0.79–1.44)
Q4 (>287 pg/mL)	1.09 (0.87–1.38)	1.21 (0.88–1.66)	1.01 (0.73–1.40)
Per 1-SD increase	1.05 (0.96–1.14)	1.08 (0.96–1.20)	1.02 (0.91–1.15)

Risk estimates for atherosclerotic cardiovascular disease per quartile and continuously per 1 standard deviation increase of plasma Aβ40. Atherosclerotic cardiovascular disease is the composite of coronary heart disease and ischaemic stroke/TIA.

(Supplemental Table S4). Results were similar after exclusion of participants with dementia (Supplemental Table S5).

3.4. Aβ40 and ASCVD: replication cohort

Compared to the main cohort, the 1467 participants in the replication sample on average were younger and displayed a different cardiovascular risk profile, with lower BMI, lower prevalence of diabetes, and less frequent use of cardiovascular preventive medication (Table 1).

During 12,240 person years of follow-up (median 9.3 years), 247 individuals developed ASVCD. Risk estimates for the association of Aβ40 with ASCVD were similar to the main cohort (model 1– HR: 1.10 [0.96–1.26], and model 2– HR: 1.06 [0.92–1.23]; Supplemental Table S5). Once again, there was no association between Aβ40 and ischaemic stroke, whereas a higher risk of CHD in the age- and sex-adjusted models attenuated after additional adjustment for cardiovascular risk factors (model 1– HR: 1.24 [1.04–1.48], and model 2– HR: 1.15 [0.96–1.39]; Supplemental Table S6).

4. Discussion

In this prospective population-based cohort, we found that higher plasma levels of amyloid-β40 are associated with subclinical atherosclerosis, notably in the coronary arteries and independent of traditional cardiovascular risk factors. Higher Aβ40 conferred a slightly highly risk of first-ever ASCVD, but this was mostly explained by differences between participants in vascular risk profile.

Clinical studies have shown that higher Aβ40 relates to an increased risk of cardiovascular mortality among patients with CHD [12,13], but associations in population-based cohorts are less profound on the basis of our findings and cardiovascular mortality in the French Three-City Study [27]. The heterogeneity could be due either to changes in Aβ40 that occur relatively late in the disease course, or differences in patient characteristics including treatment. Patients in clinical cohorts tend to be younger and more often male, and secondary preventive treatment regimens obviously differ from those for primary prevention in the general population. Stratifying our analyses by age and sex does not clearly support effect modification by demographics. In exploratory analyses, we did observe stronger associations of Aβ40 with both atherosclerosis and CHD in individuals with hypertension. Prior studies have reported that Aβ40 may increase blood pressure due to vasoconstriction or increased arterial stiffness with deposits in the tunica media [14–16]. Hypertension may thus mark a physiological effect of Aβ40, identifying a subgroup of individuals in whom Aβ40 predisposes to ASCVD. A similar interaction between Aβ40 and blood pressure has been noted for dementia risk [28], but should be confirmed or refuted by further study. In terms of prognosis, the risk estimates we observed for incident ASCVD do not support the use of plasma Aβ40 levels as a tool for refining primary ASCVD risk stratification.

The CT-based arterial calcification results that we report are in line with previously noted increases in plasma Aβ40 with coronary, carotid, and femoral artery atherosclerosis [12,29]. In contrast to the latter population-based study, findings in the current study population were independent of renal function and other potential confounders, supporting a role of Aβ40 early in the process of atherosclerosis, due for instance to their release from activated platelets in parallel to low-grade inflammation and macrophage activation [30,31]. It should be noted that Aβ40 related more strongly to atherosclerosis in the coronary

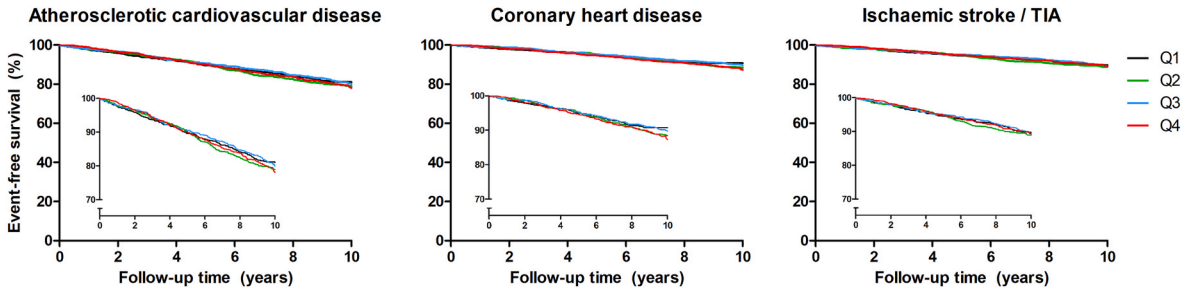

Fig. 3. Risk of first-ever atherosclerotic cardiovascular disease, coronary heart disease, and ischaemic stroke.
Adjusted survival curves by quartiles of amyloid-β40 for the main outcome of atherosclerotic cardiovascular disease, as well as segregated by coronary heart disease and ischaemic stroke. Figure insets show the same survival curves over a smaller range of the y-axis.

arteries and carotid bifurcation, compared to intracranial atherosclerosis. Plaques in the coronary arteries and at the carotid bifurcation often have a substantial non-calcified component, whereas calcification at the intracranial carotid artery siphon are generally calcified with fewer necrotic cores and intraplaque haemorrhage [32,33]. In addition, coronary and carotid bifurcation calcification occur predominantly in the tunica intima, whereas a more circular type in the medial layers is the predominant type of calcification in the intracranial carotid arteries [34]. With regard to clinical outcomes, differences in aetiology could further account for the observed difference between CHD and stroke. Large artery atherosclerosis (i.e. plaque rupture) is the leading cause of myocardial infarction in two thirds of patients [35], but accounts for only 15–20% of ischemic strokes [36]. Differentiating plaque characteristics, and large artery atherosclerosis from arterio(lo)sclerosis in smaller vessels may help clarify the role of Aβ40 in future studies.

We established various cardiometabolic determinants of Aβ40 levels in this study that can serve to avoid confounding and increase insight into the physiological properties of amyloid-β. Plasma Aβ40 is consistently reported higher with increasing age, cholesterol (be it triglycerides or HDL), and renal failure [10,12,29]. In contrast to previous studies, we observed higher levels in men than in women, and no associations with diabetes after accounting for other determinants of Aβ40 [10,29]. We additionally found positive associations with smoking, body mass index, hypertension, CRP, and haemoglobin, which were not unequivocally observed in study populations on secondary preventive treatment regimens [12]. Inverse associations with renal function could point to reduced clearance from the bloodstream [37], as well as shared aetiology or renal amyloidosis. Future studies are needed to clarify how these associations play part in the pathophysiology that links Aβ40 to atherosclerosis and CHD, as well as Alzheimer's disease.

Major strengths of this study include the high-sensitivity SiMoA Aβ40 measurement in a large, unselected population with CT-imaging and long-term follow-up for ASCVD. There are certain limitations to also take into account. First, we did not measure amyloid peptides beyond those of 40 (and 42) amino acids long, such as Aβ38, which may also play a role in cardiovascular risk [38]. Second, despite rigorous adjustment, we cannot rule out residual confounding, in particular by initiation or cessation of cardiovascular treatment in the longitudinal analyses. Third, we had no data to differentiate between aetiological subtypes of ischaemic stroke or myocardial infarction, or examine non-calcified atherosclerotic plaque characteristics. Finally, the study population consisted of predominantly white individuals (97%), potentially hampering generalisability.

In conclusion, higher plasma levels of amyloid-β40 are associated with subclinical atherosclerosis, but not risk of first-ever ASCVD after accounting for traditional cardiovascular risk factors. These findings encourage further study into the systemic involvement of Aβ40 in the process of atherosclerosis, and the potential interplay between Aβ40 and traditional risk factors in the process leading to clinical ASCVD.

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CRediT authorship contribution statement

All authors have made a substantial intellectual contribution to design of the study (AH, MAI, OHF), acquisition of data (PJK, MKI, MJGL, MWV, DB, FJW), analysis of the data (FJW), interpretation of data (FJW, SH, MK, DB, MAI), drafting the manuscript (FJW), or revising it critically for important intellectual content (SH, MJGL, MKI, MK, AH, PJK, DB, OHF, MWV, MAI). All authors approved the final version of the manuscript for publication. FJW had full access to the data in the study and takes responsibility for data integrity and accuracy of data analysis.

Declaration of competing interest

Oscar H. Franco reports grants from Nestle and Metagenics, outside the submitted work.

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Appendix A. Supplementary data

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