Neurovascular imaging markers of brain aging

Sigurdsson, S.

5

Space and location of cerebral microbleeds, cognitive decline, and dementia in the community

Manuscript based on this chapter has been published as:

Ding J, Sigurdsson S, Jonsson PV, Eiriksdottir G, Meirelles O, Kjartansson O, Lopez OL, van Buchem MA, Gudnason V, Launer LJ. Space and location of cerebral microbleeds, cognitive decline, and dementia in the community. Neurology. 2017 May 30;88(22):2089-2097.

ABSTRACT

Objectives

The number and anatomic location of cerebral microbleeds (CMBs) are visible indicators of microvascular damage on MRI, but their association with incident cognitive disease in the general population of older people is not well known.

Methods

In the longitudinal population-based AGES–Reykjavik Study, 2,602 participants aged 66-93 years free of prevalent dementia underwent brain MRI and cognitive testing of verbal memory, processing speed and executive function, at baseline and a mean of 5.2 years later. Adjudicated incident dementia cases were diagnosed according to international guidelines.

Results

In the multiple linear regression models adjusted for demographic, genetic, cardiovascular risk and other cerebrovascular MRI markers, the presence of CMBs located in deep or mixed (deep and lobar) areas, was associated with a greater decline in all three cognitive domains. Mixed CMBs were the strongest correlate for decline in memory and speed. Compared to those with no CMBs, participants with ≥3 CMBs had a steeper decline in a composite measure of global cognitive function, memory and speed. Among those with ≥3 deep or mixed CMBs, associations were strongest for memory; the association with speed was strongest in those having ≥3 strictly lobar CMBs. People with ≥3 CMBs, irrespective of their locations, had a higher incidence of all-cause dementia and vascular dementia.

Conclusions

Mixed or a higher load of CMBs, with some specificity for location, are associated with accelerated cognitive decline in older people. These findings suggest a role for hypertensive vasculopathy and the combined effect of hypertensive and cerebral amyloid angiopathy in the pathogenesis of cognitive deterioration.

INTRODUCTION

Cognitive impairment and dementia are increasingly recognized as a continuum of overlapping neurological syndromes in older people with both cerebrovascular and neurodegenerative pathology.[1-3] Exactly how the two processes interact to infer an increased risk of cognitive dysfunction is an area of intense investigation. Cerebral micorobleeds (CMBs) may provide an intriguing link between them.[4,5] Depending on location, CMBs commonly relate to the two different small vessel disease (SVD) pathologies: hypertensive vasculopathy (deep regions) and cerebral amyloid angiopathy (CAA) (lobar regions).[4]

As yet, the cognitive consequences of CMBs in the general population remain uncertain[5,6] and previous studies, mostly of cross-sectional design,[7-12] have yielded inconsistent results. While some studies[7,9] found an association between the presence of CMBs and worse cognitive performance that was strongest for deep CMBs, others[8,12] showed an association with a higher number of CMBs most strongly for lobar CMBs, and still others reported no region-specific associations.[10,11] Longitudinal studies are scant.[13,14] In particular, although mixed (deep and lobar) CMBs appear to increase the risk of developing dementia in patients with elevated vascular risk burden,[13] it remains unclear whether ostensibly non-demented older people with such pre-existing CMBs also experience more cognitive decline over time.

In the population-based AGES-Reykjavik Study, we sought to investigate whether baseline CMBs by number and location are associated with the rate of cognitive decline and incident dementia over a 5-year period. We hypothesized that people with a high load of CMBs or with mixed deep and lobar CMBs, suggesting more severe and extensive SVD, are predisposed to progressive cognitive deterioration.

METHODS

Participants

The present study was based on longitudinal data from the AGES-Reykjavik Study, which originates from the Reykjavik Study (1967–1996), as described fully elsewhere.[15] From 2002 to 2006, 5,764 surviving men and women of the Reykjavik Study cohort (born1907-1935) underwent an extensive physical, cognitive and brain MRI examination (AGES I).[15] From 2007 to 2011, there was a follow-up examination of surviving participants (AGES II). Of the 3,316 participants who attended the follow-up examination, we excluded 644 participants who had missing MRI data on CMBs at baseline and 70 participants because of

a diagnosis of prevalent dementia at baseline, missing data on either baseline cognitive status or follow-up cognitive measures. The cohort at risk of dementia thus comprised 2,602 participants (Figure 1 & Supplementary figure 1).

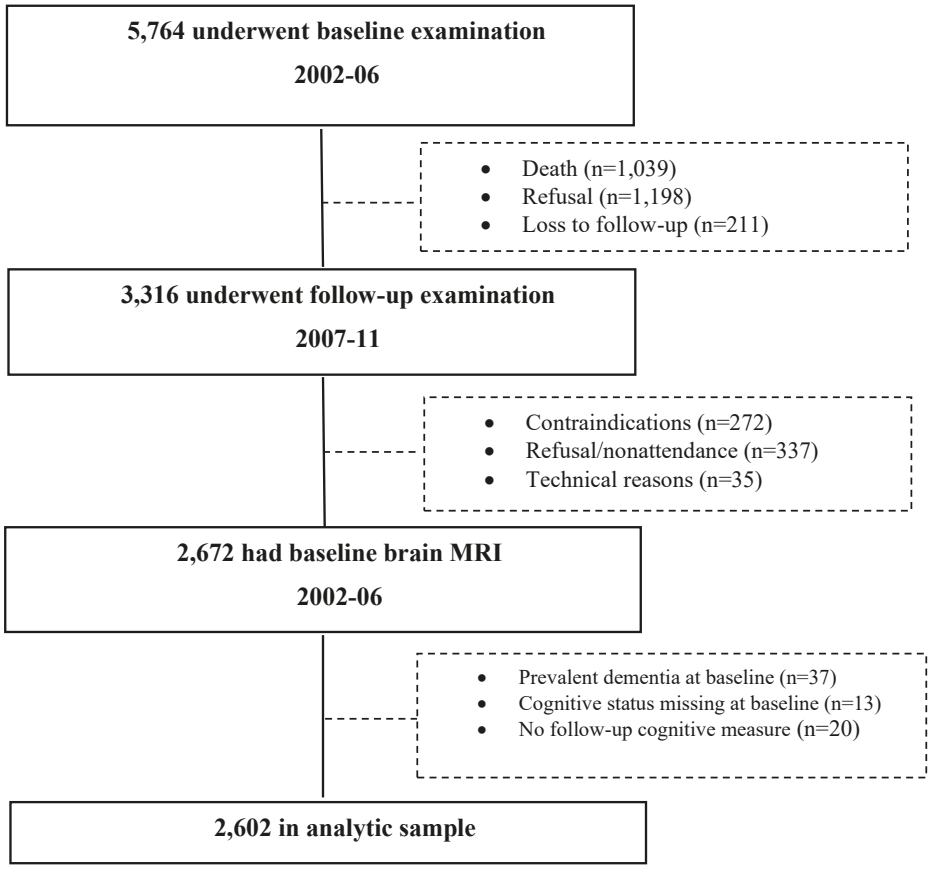

Figure 1. Study population

Standard protocol approvals, registrations, and patient consents

The study was approved by the Icelandic National Bioethics Committee (VSN 00-063), and by the National Institute on Aging Intramural Institutional Review Board. All participants gave written informed consent.

Brain MRI and rating of CMB

We acquired brain MRI scans on a single study-dedicated 1.5-T General Electrics Signa Twinspeed system (Waukesha, Wisconsin, USA).[16,17] For CMB detection, we used a 2-dimensional T2*-weighted gradient echo-type echo planar sequence (GRE-EPI) (echo time

[TE] 50 ms, repetition time [TR] 3050 ms, flip angle [FA] 90°, field of view [FOV] 220 mm, matrix 256 × 256, slice thickness 3 mm) and a proton density/T2 weighted fast spin echo (FSE) sequence (TE1, 22 ms; TE2, 90 ms; TR, 3220 ms; echo train length, 8; FA, 90°; FOV, 220 mm; matrix 256 × 256, slice thickness 3 mm).[16] Two trained radiographers evaluated CMBs on the MRI scan in terms of size and anatomical location with good intra-rater and inter-rater reliabilities for CMBs detection.[17] CMBs were defined as a focal area of signal void within the brain parenchyma that is observable on T2*-weighted GRE-EPI scans and smaller or unobservable on T2 weighted FSE scans.[16] We counted up to 30 CMBs in lobar regions (frontal, parietal, temporal, and occipital); and in deep (basal ganglia and thalamus, corpus callosum, and brain stem) or in cerebellar regions.[17] If there were > 30 CMBs, they were coded as 30. People with ≥1 CMBs confined to lobar regions were regarded as having strictly lobar CMBs and those with CMBs in a deep region, with or without coexisting lobar CMBs were regarded as having deep or mixed CMBs.[17] CMBs in the cerebellum were classified as a separate group given there is no general agreement on their presumed underlying etiology.[18,19]

Assessment of cognitive function

Participants underwent a neuropsychological test battery assessing three cognitive domains.[20] We constructed composite scores for each cognitive domain based on a theoretical grouping of tests as reported previously.[21,22] The memory composite included a modified version of the California Verbal Learning test consisting of immediate and delayed recall subtests.[20] The processing speed composite included the Digit Symbol Substitution test (DSST), the Figure Comparison Test, and the Stroop test Part I and II (word naming and color naming).[20] The executive function composite included the Digit Backwards Test and the Stroop test Part III (word-color interference).[20] We transformed the raw test scores into standardized Z scores and then averaged them across all tests for the cognitive domain. The composite score for global cognitive function was the average of the Z scores for all these domains. Higher Z-scores reflect a better cognitive performance. For each participant, we computed Z scores for both baseline and follow-up using the mean and SD of the baseline test scores. Change in cognitive functioning was calculated by subtracting the baseline Z scores for memory, processing speed, executive function, and global cognitive function from the follow-up Z scores, respectively.

Diagnosis of dementia and subtypes

Incident dementia cases were identified at follow-up based on a 3-step procedure.[7] All participants underwent the Mini-Mental State Examination and the DSST. People who were positive at screening on either test underwent additional diagnostic testing that included the Trails A and B and the Rey Auditory Verbal Learning tests.[20] Based on these tests, those who were then suspected to have dementia went further for a final

evaluation that included a proxy interview and neurologic examination.[20] The diagnosis of dementia and all subtypes was made in accordance with international criteria[23-25] (Supplementary methods) and assigned at a consensus conference by a panel of a geriatrician, neurologist, neuropsychologist, and neuroradiologist.[7]

Statistical analysis

As experimental and empirical evidence[7,8] show a higher number of CMBs is a strong indicator of underlying pathology and a single lesion is not uncommon among individuals without pathological evidence of SVD,[26] we categorized the number of CMBs into no CMBs, 1 CMB, 2 CMBs and ≥3 CMBs per person based on the skewed distribution of CMBs counts.

We first estimated the association between the CMBs count categories at baseline and subsequent cognitive decline by multiple linear regression analyses. The change scores for processing speed and global cognitive function were skewed so we transformed them with a natural logarithmic transformation. All analyses were initially adjusted for age and sex (model 1), followed by further adjustment for coil type, the time interval between the two waves of neuropsychological tests, primary education level, depressive symptomology at follow-up, hypertension, total cholesterol, use of statin, brain infarcts, white matter hyperintensity volume as percentage of total intracranial volume, and APOE ε4 carriership (model 2). We evaluated the interactions between CMBs and other covariates with respect to effects on cognitive decline by including cross-product terms of each covariate with CMBs in the fully-adjusted models. Second, we investigated the association between CMBs count categories and incident dementia. Given events for dementia, Alzheimer's disease (AD) and vascular dementia (VaD) are relatively rare (n<5) within some CMBs count categories, we merged four categories into three categories (no CMBs, 1-2 and ≥3 CMBs) and applied Fisher's exact test to examine the associations with dementia (there were not enough events to get reliable estimates of the odds ratios by using logistic regressions). All analyses were repeated according to CMBs location. To test the robustness of the results, we did several sensitivity analyses, details of which are described in Supplementary. Analyses were performed using SAS version 9.3. Associations were considered significant at the 0.05 level. Given that multiple *a priori* statistical tests were performed, the likelihood of type I errors increased. Statistical adjustment would be over-stringent as cognitive domains were correlated and we acknowledge that very small p-values tended to indicate replicable associations.[14,27]

RESULTS

Of the total 2,602 participants, the mean age at baseline was 74.6 years (SD 4.8) and 41% were men. The prevalence of CMBs was 16.8% (n=437) (median number of CMBs, 1 [range, 1-21]), of a single CMB was 12.0%, of 2 CMBs was 2.6%, and of ≥3 CMBs was 2.2% (Table 1). Compared to participants with no CMBs, the other three CMBs groups were older, more likely to be male, APOE ε4 allele carriers and hypertensive, more likely to use statin and antithrombotic medications, have lower average total cholesterol level and higher total brain volume, and have cardiovascular disease as well as ischemic vascular lesions on brain MRI.

Among participants with CMBs (n=437), 71.2% (n=311) had CMBs in a strictly lobar location, 13.3% (n=58) had CMBs in deep or mixed locations (includes those with mixed CMBs in both deep and lobar locations, n=25), and 15.5% (n=68) had CMBs in the cerebellum. Among those with ≥3 CMBs (n=58), 55.2% (n=32) had strictly lobar CMBs, 27.6% (n= 16) had deep or mixed CMBs and 17.2% (n=10) had cerebellar CMBs.

Prevalent CMBs at baseline and cognitive decline

In the fully-adjusted models, presence of deep or mixed CMBs was significantly associated with a steeper decline in a composite measure of global cognitive function and specifically, in performance on all 3 cognitive domains including memory, information processing speed and executive function (Table 2). Mixed CMBs were most strongly associated with a decline in memory and speed (Figure 2 & Supplementary table 1). Presence of strictly lobar CMBs was not associated with cognitive decline. Compared to participants with no CMBs, those with ≥3 CMBs had a greater decline in, separately, global cognition, memory and speed. Associations in those with ≥3 CMBs in deep or mixed locations were strongest for memory. Participants with ≥3 CMBs in a strictly lobar location had significantly greater decline in processing speed. No association was observed for cerebellar CMBs with respect to CMBs count categories or location (Supplementary table 2).

Prevalent CMBs at baseline and incident dementia

Over a mean follow-up of 5.2 years (SD 0.2), 4.5% (n=119) participants developed all-cause dementia, of whom 68.9% (n=82) had AD, 14.3% (n=17) had VaD and 3.4% (n=4) had both possible AD and possible VaD. The remaining 16 cases were attributed to other subtypes, such as dementia in Parkinson disease and Lewy body dementia. The cumulative incidence (%) of dementia and its subtypes according to CMBs count and location was shown in Table 3.

Table 1. Characteristics of the study population (n=2,602) according to the count categories of cerebral microbleeds (CMBs) in AGES I.

	CMBs count				
	No CMBs (n=2,165)	1 CMB (n=311)	2 CMBs (n=68)	≥3 CMBs (n=58)	P for trend*
Age, years	74.5 (4.8)	75.4 (4.7)	75.4 (4.4)	75.4 (4.7)	0.001
Men	38.5 (834)	51.8(161)	55.9(38)	63.8(37)	<0.001
Primary education only	19.9(430)	23.3(72)	11.8(8)	19.3(11)	0.555
MMSE score	28.0(26.0-29.0)	28.0(26.0-29.0)	27.0(26.0-29.0)	27.0(26.0-29.0)	0.619
Depressive symptomology at baseline	4.8 (99)	4.5(13)	6.0(4)	7.1(4)	0.423
Depressive symptomology at follow-up	6.6(141)	4.9(15)	6.1(4)	10.3(6)	0.961
APOE ε4 allele carriers	25.7(555)	25.1(78)	38.2(26)	36.2(21)	0.014
Cardiovascular risk factors/ disease					
Body mass index (kg/m^2)	27.3 (4.2)	27.1 (3.7)	26.3 (3.9)	26.7 (3.3)	0.070
Current smoker	10.7(230)	9.7(30)	14.7(10)	10.3(6)	0.520
Systolic blood pressure, mmHg	140.5 (19.3)	143.2 (20.9)	143.7(21.9)	146.7 (21.5)	0.004
Diastolic blood pressure, mmHg	74.1(9.2)	74.3(9.9)	75.9(8.6)	75.4(8.9)	0.035
Hypertension	76.3 (1,651)	82.3(256)	86.8 (59)	89.7(52)	0.001
Type 2 diabetes	9.1 (197)	9.4(29)	7.4(5)	17.2(10)	0.180
Total cholesterol, mmol	5.7(1.1)	5.4(1.1)	5.3(1.1)	5.3(1.0)	<0.001
History of coronary heart disease	15.7(339)	28.3(88)	32.4(22)	36.2(21)	<0.001
History of stroke	2.5(53)	3.9(12)	7.4(5)	8.6(5)	0.001
Medication use					
Use of blood pressure lowering medication	58.5(1267)	65.6(204)	69.1(47)	77.6(45)	<0.001
Use of salicylate/anticoagulants	24.3(469)	32.7(90)	36.5(23)	37.5(21)	<0.001
Statin	21.5(466)	33.1(103)	39.7(27)	36.2(21)	<0.001
Brain MRI markers					
Cerebral infarcts	28.8(623)	38.6(120)	42.7(29) 53.5(31)		<0.001
White matter hyperintensitity volume, ml	11.2(6.4-20.1)	13.9(7.1-26.5)	17.3(10.1-38.6) 19.9(11.1-33.6)		<0.001
Total brain parenchyma volume, mL	1092.7(102.7)	1105.2(103.9)	1096.2(108.6) 1124.1(108.6)		0.0006

Data are presented as percentage of participants (n), mean (standard deviation) or median (interquartile range). *Age-adjusted

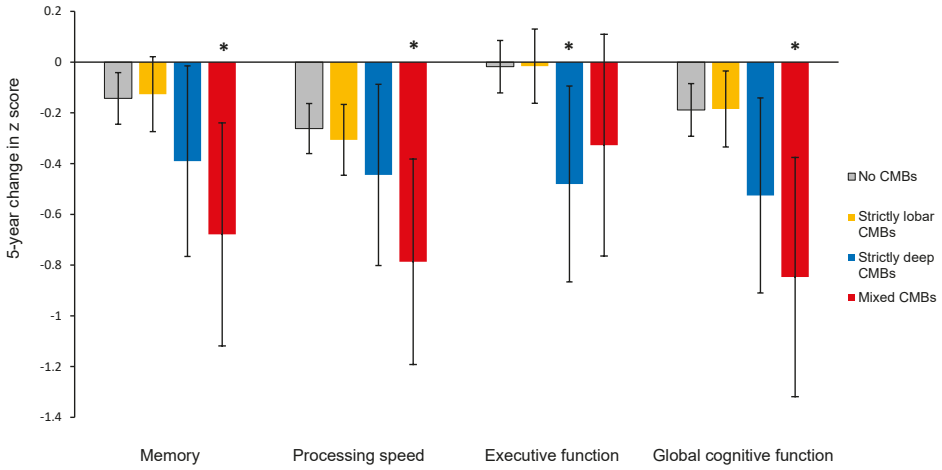

Figure 2. Multivariable-adjusted 5-year change in cognitive domains for microbleeds locations. Error bars represent 95% confidence intervals. Adjusted for age, sex, follow-up interval, coil type, primary education, depression at follow-up, hypertension, total cholesterol, use of lipid-lowering medication, presence of brain infarcts, measure of white matter hyperintensity volume expressed as percentage of total intracranial volume, and APOE e4 carriership.*p , 0.05.

Table 2. Association (β regression coefficient [95%confidence interval]) of prevalent cerebral microbleeds (CMBs) with 5-year cognitive change[•] (decline in Z scores) between baseline and follow-up in those who were free of prevalent dementia at baseline (n=2,561) [†]

	Memory		Processing speed		Working memory /executive function		Global cognitive function	
	Model 1 [‡]	Model 2 [§]	Model 1[‡]	Model 2[§]	Model 1[‡]	Model 2[§]	Model 1[‡]	Model 2[§]
CMBs, by number								
No CMBs (n=2129)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)
1 CMB (n=307)	0.09 (-0.04 to 0.21)	0.08 (-0.05 to 0.21)	0.03 (-0.09 to 0.15)	0.02 (-0.10 to 0.14)	-0.03 (-0.16 to 0.09)	-0.02 (-0.15 to 0.10)	0.03 (-0. 06 to 0.20)	0.06 (-0. 07 to 0.19)
2 CMBs (n=67)	-0.24 (-0.49 to 0.01)	-0.17 (-0.42 to 0.09)	-0.22 (-0.46 to 0.03)	-0.16 (-0.41 to 0.08)	-0.11 (-0.36 to 0.14)	-0.08 (-0.33 to 0.18)	-0.29 (-0.55 to -0.04)	-0.21 (-0.47 to 0.04)
≥3 CMBs (n=58)	-0.32 (-0.59 to -0.05)	-0.29 (-0.57 to -0.01)	-0.37 (-0.63 to -0.11)	-0.31 (-0.57 to -0.05)	-0.20 (-0.50 to 0.08)	-0.18 (-0.46 to 0.10)	-0.38 (-0.66 to -0.09)	-0.34 (-0.63 to -0.05)
P for trend	0.061	0.136	0.009	0.038	0.104	0.192	0.018	0.047
Overall CMBs (n=432)	-0.02 (-0.13 to 0.09)	-0.008 (-0.12 to 0.10)	-0.06 (-0.17 to 0.04)	-0.05 (-0.15 to 0.06)	-0.07 (-0.18 to 0.04)	-0.05 (-0.16 to 0.06)	-0.05 (-0.16 to 0.07)	-0.03 (-0.15 to 0.08)

Table 2. Association (β regression coefficient [95%confidence interval]) of prevalent cerebral microbleeds (CMBs) with 5-year cognitive change[*] (decline in Z scores) between baseline and follow-up in those who were free of prevalent dementia at baseline (n=2,561) [†] (continued)

	Memory		Processing speed		Working memory /executive function		Global cognitive function	
	Model 1[‡]	Model 2[§]	Model 1[‡]	Model 2[§]	Model 1[‡]	Model 2[§]	Model 1[‡]	Model 2[§]
CMBs, by location & number								
1 strictly lobar CMB (n=239)	0.09 (-0.05 to 0.23)	0.09 (-0.06 to 0.23)	0.007 (-0.12 to 0.14)	0.00 (-0.13 to 0.14)	0.01 (-0.13 to 0.15)	0.03 (-0.12 to 0.17)	0.09 (-0.05 to 0.24)	0.08 (-0.07 to 0.23)
2 strictly lobar CMBs (n=35)	-0.20 (-0.54 to 0.13)	-0.16 (-0.49 to 0.17)	-0.06 (-0.39 to 0.27)	-0.03 (-0.36 to 0.30)	-0.05 (-0.40 to 0.29)	-0.03 (-0.38 to 0.32)	-0.20 (-0.54 to 0.14)	-0.14 (-0.48 to 0.20)
≥3 strictly lobar CMBs (n=32)	-0.26 (-0.62 to 0.10)	-0.25 (-0.62 to 0.11)	-0.51 (-0.84 to -0.17)	-0.47 (-0.81 to -0.12)	-0.12 (-0.49 to 0.24)	-0.12 (-0.50 to 0.25)	-0.35 (-0.72 to 0.02)	-0.34 (-0.72 to 0.03)
P for trend	0.414	0.488	0.040	0.069	0.638	0.776	0.278	0.334
Overall strictly lobar CMBs (n=306)	0.01 (-0.12 to 0.14)	0.02 (-0.11 to 0.15)	-0.02 (-0.11 to 0.16)	0.03 (-0.11 to 0.16)	0.01 (-0.13 to 0.16)	0.005 (-0.13 to 0.14)	0.006 (-0.13 to 0.14)	0.02 (-0.11 to 0.15)
1 deep and/ or mixed CMB (n=24)	-0.11 (-0.53 to 0.31)	-0.17 (-0.60 to 0.26)	-0.13 (-0.53 to 0.26)	-0.21 (-0.61 to 0.20)	-0.49 (-0.92 to -0.05)	-0.52 (-0.97 to- 0.07)	-0.23 (-0.66 to 0.20)	-0.30 (-0.74 to 0.14)
2 deep and/ or mixed CMBs(n=18)	-0.53 (-1.02 to -0.04)	-0.35 (-0.86 to 0.16)	-0.55 (-1.01 to -0.10)	-0.41 (-0.89 to 0.06)	-0.40 (-0.87 to 0.08)	-0.33 (-0.83 to 0.16)	-0.76 (-1.25 to -0.26)	-0.59 (-1.11 to -0.08)
≥3 deep and/or mixed CMBs(n=16)	-0.77 (-1.32 to -0.23)	-0.69 (-1.24 to -0.14)	-0.55 (-1.02 to -0.08)	-0.44 (-0.91 to 0.04)	-0.33 (-0.85 to 0.19)	-0.27 (-0.80 to 0.25)	-0.66 (-1.24 to -0.08)	-0.55 (-1.13 to 0.03)
P for trend	0.0006	0.0042	0.0012	0.0091	0.012	0.030	0.0002	0.0020
Overall deep or mixed CMBs (n=58)	-0.42 (-0.71 to -0.13)	-0.35 (-0.63 to- 0.07)	-0.40 (-0.68 to -0.13)	-0.30 (-0.56 to -0.04)	-0.41 (-0.70 to -0.11)	-0.37 (-0.65 to -0.08)	-0.52 (-0.82 to -0.21)	-0.43 (-0.72 to- 0.13)

[*] Cognitive change was defined as the difference between composite cognitive z scores at follow-up and those at baseline; a negative change score indicated cognitive decline; [†] 41 individuals out of 2,602 participants who had missing data on all three cognitive domain measures in AGESII were excluded from cognitive decline analysis [‡] Model 1 was adjusted for age and sex [§] Model 2 was further adjusted for follow-up time interval, coil type, primary education, depression at follow-up, hypertension, total cholesterol, use of lipid-lowering medication, the presence of brain infarcts, measure of white matter hyperintensity volume expressed as percentage of total intracranial volume and Apolipoprotein E ε4 carriership.

Table 3. Association of prevalent cerebral microbleeds (CMBs) with incident dementia (n=2,601)*

	Dementia (n=119)			Alzheimer disease (n=86)†			Vascular dementia (n=21)†		
	No.	%	Fisher' exact test Two-tailed p-value	No.	%	Fisher' exact test Two-tailed p-value	No.	%	Fisher' exact test Two-tailed p-value
CMBs, by number									
No CMBs (n=2164)	99	4.6%	(reference)	74	3.5%	(reference)	15	0.7%	(reference)
1-2 CMBs (n=379)	12	3.2%	0.920	9	2.4%	0.893	2	0.5%	0.751
≥3CMBs (n=58)	8	13.8%	**0.006**	3	5.7%	0.285	4	7.4%	**0.001**
Overall CMBs (n=437)	20	4.6%	1.00	12	2.8%	0.559	6	1.4%	0.149
Strictly lobar CMBs									
1-2 strictly lobar CMB(n=279)	8	2.9%	0.936	6	2.2%	0.912	-	-	-
≥3 strictly lobar CMBs (n=32)	5	15.6%	**0.015**	3	10.0%	0.088	2	6.9%	**0.022**
Overall strictly lobar CMBs (n=311)	13	4.2%	0.884	9	2.9%	0.737	2	0.7%	1.00
Deep or mixed CMBs									
1-2 deep and/ or mixed CMB(n=42)	2	4.8%	0.718	2	4.8%	0.656	1	2.4%	0.269
≥3 deep and/ or mixed CMBs (n=16)	3	18.8%	**0.036**	-	-	-	2	13.3%	**0.006**
Overall deep and/or mixed CMBs (n=58)	5	8.6%	0.192	2	3.6%	0.716	3	5.4%	**0.010**

* 1 individual out of 2,602 participants who had missing dementia status in AGESII was excluded from dementia analysis
† The number included 4 cases with both possible Alzheimer's disease and possible vascular dementia.

The presence of deep or mixed CMBs was associated with a higher incidence of VaD. Compared to those with no CMBs, participants with ≥3 CMBs had a higher incidence of all-cause dementia and VaD. Similar association patterns were observed for those with ≥3 strictly lobar CMBs or with ≥3 deep or mixed CMBs. Cerebellar CMBs revealed no significant associations (Supplementary table 2).

Sensitivity analyses

The association between mixed CMBs and cognitive decline persisted for memory and global cognitive function, while the association with processing speed lost statistical significance after further controlling for median CMBs count (Supplementary table 3). Additional adjustment for use of anticoagulants/salicylate, brain atrophy or prevalent stroke, or analyses with imputed covariate values generated similar results with respect to the associations between CMBs and cognitive decline. When including participants with baseline prevalent dementia (n=37) in analysis, we observed similar slopes on cognitive decline (Supplementary table 4).

DISCUSSION

In a community-based sample of older people free of prevalent dementia at baseline, we found that mixed CMBs or ≥3 CMBs, with some specificity for location, are associated with accelerated cognitive decline. These results were independent of education level, depression, APOE ε4 carriership, cardiovascular risk factors and other MRI markers of cerebrovascular disease including brain atrophy. Further, there is suggestive evidence of a higher rate of incident dementia and VaD in participants with ≥3 CMBs.

Previously, two small longitudinal studies[13,28] among selected individuals or clinic-based patients, have reported on an association between multiple or mixed CMBs and cognitive decline or dementia. Recently, another large population-based study showed an association of a high number of lobar CMBs with cognitive decline in executive function and processing speed.[14] They did not find an association with deep or mixed CMBs. However, compared to the AGES-Reykjavik study, the cohort had a lower mean age and fewer cardiovascular risk factors including hypertension and duration of hypertension, which may moderate the associations we found. The current results thus add significantly to our understanding of the cognitive consequences of CMBs in a large well described community-based sample of older adults free of dementia. Our findings are consistent with the hypothesis that CMBs, especially mixed or a high load of CMBs, exert deleterious effects on cognitive decline, eventually leading to full-blown dementia.

Although the precise underlying mechanisms of the observed associations between, on the one hand, mixed or a higher number (≥3) of CMBs and, on the other, accelerated cognitive decline have not been established, there are several possible explanations. CMBs may reflect focal damage of brain tissue as well as concomitant microstructural damage of the surrounding tissue (e.g., microinfarcts or gliosis).[29] As a result, they may disrupt connections of functionally important cortical and subcortical tracts that are critical for

cognitive processes, ultimately leading to damage of neural networks and interfering with cognition.[29] Alternatively, CMBs are more likely to imply more generalized microvascular damage that disrupts the blood-brain barrier or causes hypoxia.[13,29] In this scenario, CMBs are only the tip of the iceberg of SVD and mixed or a higher number of CMBs may thus indicate more extensive and severe subclinical microvascular damage.

We found that the associations with cognitive decline differed according to the spatial location of CMBs, and thus possibly differed with underlying vasculopathy. Our results suggest that ≥3 CMBs in strictly lobar regions, presumably resulting from CAA, were related to a faster decline in processing speed. On the other hand, participants with ≥3 strictly lobar CMBs had highest prevalence of cerebral infarcts and largest volume of white matter hyperintensities at baseline, compared to those with none, 1 or 2 strictly lobar CMBs in the present study. Thus, this finding of impaired speed associated with a relatively high load of lobar CMBs, shown previously,[8,12] suggests that the vascular damage and ischemia[30] caused by (or predisposing to) CAA[31] can reflect overall vascular damage.

Presence of CMBs in deep or mixed regions, primarily resulting from hypertensive arteriopathy, was associated with more rapid decline in all cognitive domains, and in particular, our results suggest that ≥3 CMBs in deep or mixed regions were associated with fastest decline in verbal memory. The associations with speed and executive function are consistent with the hypothesis that cerebral microvascular damage preferentially affects white matter and subcortical grey matter, with disruption of integrity of frontal-subcortical circuits.[22,32] Our finding of deep or mixed CMBs in association with verbal memory, independent of brain atrophy, might suggest disruption of thalamic nuclei, which are involved in storage and short-term memory,[32] and is consistent with memory impairment, the hallmark of AD, also being present in vascular-related cognitive impairment.[31]

Of note, mixed CMBs were most strongly associated with a decline in memory and global cognitive function compared to CMBs either in strictly lobar or in strictly deep locations. These findings may also underscore the role of interplay between hypertensive vasculopathy and CAA in the pathogenesis of cognitive decline. It is possible that vascular amyloid-ß deposition impairs reactivity of cerebral microvasculature and causes functional loss with ischemic and hemorrhagic lesions.[6] In parallel, hypertensive damage to small vessels impairs arterial pulsation and results in failure of perivascular drainage, which reduces clearance of amyloid-ß and leads to further deposition of amyloid-ß in vessel walls.[6,33]

Apart from cognitive decline, our findings pointed in the direction of adverse effects of ≥3 CMBs on dementia incidence, particularly for VaD. However, the relatively small number of sub-typed dementia events limited our ability to conduct in-depth, adjusted statistical analysis. Further meta-analysis of individual population-based studies with adequate statistical power is warranted.

Major strengths of the present study include the longitudinal design, the large population-based sample of old people who were not demented at baseline and followed for 5 years on average, the use of a comprehensive cognitive battery and standard MRI, reliable assessment of baseline CMBs, as well as the extensive characterization of participants that enabled us to control for a series of potential confounders including other MRI markers of cerebrovascular disease.

There are several issues however that affect the interpretation of these data. Although we administered a wide range of neuropsychological tests to assess each particular cognitive domain, there is still a chance to underestimate the complex executive functions, which is the leading presentation of cognitive decline in vascular disease. Moreover, pure VaD is relatively rare and the current diagnostic criteria for VaD have low sensitivity.[25,34] On the other hand, we cannot fully rule out the possibility that the presence of CMBs may have unconsciously biased the diagnosis towards VaD due to lack of blinding to CMBs findings. The categorization of CMBs by location is presumably suggestive of specific underlying small vessel pathology. Although in line with current research and clinical practice, the categorization does not accurately reflect the multifactorial nature of CMBs[35] and as such, may be an oversimplification of underlying cognition-related pathology of location-specific CMBs. Further, the relatively lower field strength and spatial resolution of the MRI scanner we used may have underestimated the number of CMBs and affected assessment of other MRI lesions. Finally, people who were included in the analysis were younger, more educated, had better vascular health and cognitive profiles at baseline than those who were excluded. If those excluded were similarly affected by CMBs as those included in the analysis, our results may be underestimated.

Our findings support the hypothesis that CMBs are important indicators of a microvascular contribution to cognitive impairment in older people and highlight the role for hypertensive vasculopathy and the combined effect of hypertensive and cerebral amyloid angiopathy in cognitive deterioration. Multimodal neuroimaging assessments of brain metabolism, fiber tract integrity and amyloid burden, may help explain the underlying pathophysiology and integration of vascular and neurodegenerative lesions of CMBs and associated cognitive impairment.

REFERENCES

1. Iadecola C. The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. *Acta Neuropathol.* 2010;120: 287-296.
2. Fotuhi M, Hachinski V, Whitehouse PJ. Changing perspectives regarding late-life dementia. *Nat Rev Neurol.* 2009; 5: 649-658.
3. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke*. 2011; 42: 2672-2713.
4. Greenberg SM, Vernooij MW, Cordonnier C, et al. Cerebral microbleeds: a guide detection and interpretation. *Lancet Neurol.* 2009; 8:165-174.
5. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain*. 2011; 134: 335-344.
6. Charidimou A, Werring DJ. Cerebral microbleeds and cognition in cerebrovascular disease: an update. *J Neurol Sci.* 2012; 322: 50-55.
7. Qiu C, Cotch MF, Sigurdsson S, et al. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology.* 2010;75: 2221-2228.
8. Poels MM, Ikram MA, van der Lugt A, et al. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. *Neurology*. 2012; 78:326-333.
9. Yakushiji Y, Noguchi T, Hara M, et al. Distributional impact of brain microbleeds on global cognitive function in adults without neurological disorder. Stroke. 2012;43:1800 1805.
10. Hilal S, Saini M, Tan CS, et al. Cerebral microbleeds and cognition: the epidemiology of dementia in Singapore study. *Alzheimer Dis Assoc Disord.* 2014; 28:106-112.
11. Takashima Y, Mori T, Hashimoto M, et al. Clinical correlating factors and cognitive function in community-dwelling healthy subjects with cerebral microbleeds. *J Stroke Cerebrovasc Dis.* 2011; 20:105-110.
12. Meier IB, Gu Y, Guzaman VA, et al. Lobar microbleeds are associated with a decline in executive functioning in older adults. Cerebrovasc Dis. 2014; 38:377-383.
13. Miwa K, Tanaka M, Okazaki S, et al. Multiple or mixed cerebral microbleeds and dementia in patients with vascular risk factors. Neurology. 2014; 12; 83:646-653.
14. Akoudad S, Wolters FJ, Viswanathan A, et al. Association of cerebral microbleeds with cognitive decline and dementia. JAMA Neurol. 2016; 73: 934-943.
15. Harris TB, Launer LJ, Eiriksdottir G, et al. Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. *Am J Epidemiol.* 2007;165:1076-1087.
16. Sveinbjornsdottir S, Sigurdsson S, Aspelund T, et al. Cerebral microbleeds in the population based AGES-Reykjavik study: prevalence and location. *J Neurol Neurosurg Psychiatry*. 2008;79: 1002-1006.
17. Ding J, Sigurdsson S, Garcia M, et al. Risk factors associated with incident cerebral microbleeds according to location in older people: The Age, Gene/Environment Susceptibility (AGES)-Reykjavik study. *JAMA Neurol*. 2015;72: 682-688.
18. Itoh Y, Yamada M, Hayakawa M, Otomo E, Miyatake T. Cerebral amyloid angiopathy: a significant cause of cerebellar as well as lobar cerebral hemorrhage in the elderly. *J Neurol Sci.* 1993;116: 135-141.
19. De Reuck JL, Deramecourt V, Auger F, et al. The significance of cortical cerebellar microbleeds and microinfarcts in neurodegenerative and cerebrovascular diseases. A post-mortem 7.0-tesla magnetic resonance study with neuropathological correlates. *Cerebrovasc Dis.* 2015; 39:138-143.

20. Saczynski JS, Sigurdsson S, Jonsdottir MK, et al. Cerebral infarcts and cognitive performance: importance of location and number of infarcts. Stroke. 2009; 40: 677-682.
21. Prins ND, van Dijk EJ, den Heijer T, et al. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain*. 2005;128: 2034-2041.
22. Wilson RS, Mendes De Leon CF, et al. Participation in cognitively stimulating activities and risk of incident Alzheimer disease. *JAMA*. 2002; 287:742-748.
23. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34: 939-944.
24. Chui HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. *Neurology*. 1992; 42: 473-480.
25. Lopez OL, Kuller LH, Becker JT, et al. Classification of vascular dementia in the Cardiovascular Health Study Cognition Study. *Neurology*. 2005; 64:1539-1547.
26. De Reuck J, Deramecourt V, Cordonnier C, Leys D, Maurage CA, Pasquier F. The impact of cerebral amyloid angiopathy on the occurrence of cerebrovascular lesions in demented patients with Alzheimer features: a neuropathological study. *Eur J Neurol*. 2011; 18: 913-918.
27. Ding J, Strachan MW, Fowkes FG, et al. Association of retinal arteriolar dilatation with lower verbal memory: the Edinburgh Type 2 Diabetes Study. *Diabetologia*. 2011;54:1653-1662.
28. Chiang GC, Cruz Hernandez JC, Kantarci K, Jack CR Jr, Weiner MW; Alzheimer's Disease Neuroimaging Initiative. Cerebral microbleeds, CSF p-tau, and cognitive decline: significance of anatomic distribution. *AJNR Am J Neuroradiol*. 2015; 36:1635-1641.
29. Werring DJ, Gregoire SM, Cipolotti L. Cerebral microbleeds and vascular cognitive impairment. *J Neurol Sci*. 2010; 299:131-135.
30. Park JH, Seo SW, Kim C, et al. Pathogenesis of cerebral microbleeds: In vivo imaging of amyloid and subcortical ischemic small vessel disease in 226 individuals with cognitive impairment. *Ann Neurol.* 2013; 73: 584-593.
31. Resnick SM, Sojkova J, Zhou Y, et al. Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by 11C PiB. *Neurology*. 2010; 74: 807-815.
32. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and the risk of dementia and cognitive decline. *N Engl J Med*. 2003; 348:1215-1222.
33. Tarasoff-Conway JM, Carare RO, Osorio RS, et al. Clearance systems in the brain-implications for Alzheimer disease. *Nat Rev Neurol*. 2015; 11: 457-470.
34. Knopman DS, DeKosky ST, Cummings JL, et al. Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology.* 2001;56:1143-1153.
35. Akoudad S, Portegies ML, Koudstaal PJ, et al. Cerebral microbleeds are associated with an increased risk of stroke: the Rotterdam study. *Circulation*. 2015; 132: 509-516.

SUPPLEMENTARY MATERIAL

Supplementary methods

Other brain MRI measures

Cerebral infarcts were defined as parenchymal defects (1) with a signal intensity equal to cerebrospinal fluid on all pulse sequences (i.e. fluid attenuated inversion recovery sequence [FLAIR], T2-weighted and proton density-weighted sequences); (2) with a minimal diameter of 4 mm excepting for infarcts in the cerebellum that had no size criteria; and (3) being surrounded by an area of high signal intensity on FLAIR images.[1] White matter hyperintensities were defined as hyperintense lesions compared to the signal intensity of normal-appearing white matter on both T2-weighted and FLAIR images. White matter hyperintensities volume and total brain volume (a marker of brain parenchymal atrophy) were computed in an automatic manner with an algorithm based on the Montreal Neurological Institute pipeline and were expressed as the percentage of total intracranial volume.[2,3] From October 7, 2003 and onwards, we upgraded the four-channel phased array head cap coil to the eight-channel during the study. One third participants at baseline had the four-channel head coil. A study of repeated scans with 2 coils showed little impact on brain volumetric measurements.

Diagnosis of dementia and subtypes

Dementia was diagnosed according to the guidelines of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV).[4] Dementia cases were classified in accordance with the criteria for Alzheimer's disease published by the National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association [4,5] and the criteria for vascular dementia published by the State of California Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC).[6]

Assessments of covariates

Education level (primary vs. secondary/college/university education) and cigarette smoking history (current vs. former/never) were acquired through questionnaire.[7] High depressive symptomology was defined as a score ≥6 on the 15-item Geriatric Depression Scale (score range, 0 to 15).[1] Current use of medications (e.g., statin and anticoagulants/salicylate) was assessed from vials brought to the clinic.[3] Body mass index was calculated as measured weight (kg) divided by height squared (m^2).[8] Fasting total cholesterol was determined on a Hitachi 912 instrument using comparable enzymatic procedures (Roche Diagnostics, Mannheim, Germany).[7] Blood pressures were measured in recumbent position with a standard mercury sphygmomanometer, and the average of two consecutive readings was used.[7] Hypertension was defined as systolic blood pressure

≥140 mmHg or diastolic blood pressure≥90 mm Hg or use of blood pressure-lowering medications.[7] Type 2 diabetes was defined as a self-reported history of diabetes, use of blood glucose-lowering medications or a fasting blood glucose level ≥7.0 mmol/l.[7] Prevalent stroke and coronary heart disease was ascertained from hospital medical records or the registry of adjudicated cases on stroke. Apolipoprotein E genotyping was performed using standard DNA amplification and restriction isotyping.[7]

Analytic cohort

Participants excluded from the present analysis, as compared with those who met inclusion criteria, were more likely to be older and APOE ε4 allele carriers, to have depressive symptomology, hypertension, diabetes and cardiovascular disease, and were more often treated with salicylate/anticoagulants.

Statistical analysis

To explore the effect of systematic inter-location difference in CMBs numbers on location-specific associations, we compared each location category with no CMBs group while additionally accounting for CMBs count. We repeated analyses additionally adjusting for use of anticoagulants/salicylate, brain atrophy or prevalent stroke at baseline. We reran the analyses on the association with cognitive decline after inclusion of participants with prevalent dementia at baseline. Missing covariate data (≤2.2%) were either included as the reference group (education, depression and APOE ε4) or replaced with median (white matter hyperintensities volume) in analysis.

Supplementary table 1. Association of prevalent cerebral microbleeds (CMBs) in strictly deep or in mixed locations with cognitive change (decline in Z scores) and incident dementia

	Memory*		**Processing speed***		**Working memory /executive function***		**Global cognitive function***	
	Model 1†	Model 2‡	Model 1†	Model 2‡	Model 1†	Model 2‡	Model 1†	Model 2‡
	β (95%CI)		β (95%CI)		β (95%CI)		β (95%CI)	
No CMBs (n=2129)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)
Overall strictly deep CMBs (n=33)	-0.21 (-0.57 to 0.15)	-0.25 (-0.61 to 0.12)	-0.14 (-0.49 to 0.20)	-0.18 (-0.53 to 0.16)	**-0.45 (-0.82 to -0.09)**	**-0.46 (-0.84 to -0.09)**	0.13 (-0. 18 to 0.44)	0.12 (-0.19 to 0.44)
Overall mixed CMBs (n=25)	**-0.70 (-1.12 to -0.27)**	**-0.54 (-0.98 to-0.10)**	**-0.68 (-1.08 to -0.29)**	**-0.52 (-0.93 to -0.12)**	-0.37 (-0.79 to 0.04)	-0.31 (-0.75 to 0.12)	**-0.84 (-1.29 to -0.38)**	**-0.66 (-1.13 to-0.19)**
	Dementia (n=119)			**Alzheimer disease (n=86)**			**Vascular dementia (n=21)**	
	No.	%	Fisher' exact test Two-tailed p-value	No.	%	Fisher' exact test Two-tailed p-value	No.	%	Fisher' exact test Two-tailed p-value
No CMBs (n=2164)	99	4.6%	(reference)	74	3.5%	(reference)	15	0.7%	(reference)
Overall strictly deep CMBs (n=23)	2	6.1%	0.662	2	6.1%	0.322	1	3.1%	0.217
Overall mixed CMBs (n=25)	3	12.0%	0.107	-	-	-	2	8.3%	**0.015**

*Cognitive change was defined as the difference between composite cognitive z scores at follow-up and those at baseline; a negative change score indicated cognitive decline;
† Model 1 was adjusted for age and sex
‡ Model 2 was further adjusted for follow-up time interval, coil type, primary education, depression at follow-up, hypertension, total cholesterol, use of lipid-lowering medication , the presence of brain infarcts, measure of white matter hyperintensity volume expressed as percentage of total intracranial volume and Apolipoprotein E ε4 carriership.

Supplementary table 2. Association of prevalent cerebral microbleeds (CMBs) in cerebellum with cognitive changea (decline in Z scores) and incident dementia

	Memory*		**Processing speed***		**Working memory /executive function***		**Global cognitive function***	
	Model 1‡	Model 2§	Model 1‡	Model 2§	Model 1‡	Model 2§	Model 1‡	Model 2§
	β (95%CI)		β (95%CI)		β (95%CI)		β (95%CI)	
No CMBs (n=2129)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)
1 cerebellar CMB(n=44)	0.19 (-0.12 to 0.50)	0.18 (-0.14 to 0.49)	0.20 (-0.08 to 0.49)	0.20 (-0.09 to 0.48)	-0.06 (-0.36 to 0.25)	-0.05 (-0.36 to0.26)	0.13 (-0. 18 to 0.44)	0.12 (-0.19 to 0.44)
2 cerebellar CMBs(n=14)	0.03 (-0.52 to 0.57)	0.07 (-0.48 to 0.61)	-0.20 (-0.69 to 0.29)	-0.24 (-0.73 to 0.25)	0.10 (-0.44 to 0.64)	0.11 (-0.43 to0.65)	0.04 (-0. 51 to 0.60)	0.05 (-0.50 to 0.61)
≥3 cerebellar CMBs (n=10)	0.14 (-0.51 to 0.80)	0.14 (-0.46 to 0.85)	0.30 (-0.28 to 0.88)	0.28 (-0.30 to 0.86)	-0.20 (-0.85 to 0.44)	-0.18 (-0.83 to 0.47)	-0.08 (-0.76 to 0.60)	-0.02 (-0.70 to 0.65)
P for trend	0.358	0.295	0.357	0.447	0.698	0.770	0.739	0.672
Overall cerebellar CMBs (n=68)	0.15 (-0.10 to 0.41)	0.14 (-0.11 to 0.40)	0.16 (-0.08 to 0.40)	0.15 (-0.09 to 0.39)	-0.07 (-0.32 to 0.18)	-0.08 (-0.33 to 0.17)	0.08 (-0.18 to 0.33)	0.06 (-0.20 to 0.32)
	Dementia (n=119)			**Alzheimer disease (n=86)**		**Vascular dementia (n=21)**		
	No.	%	Fisher' exact test Two-tailed p-value	No.	%	Fisher' exact test Two-tailed p-value	No.	%	Fisher' exact test Two-tailed p-value
No CMBs (n=2164)	99	4.6%	(reference)	74	3.5%	(reference)	15	0.7%	(reference)
Overall cerebellar CMBs (n=68)†	2	2.9%	0.767	1	1.5%	0.727	1	1.5%	0.399

*Cognitive change was defined as the difference between composite cognitive z scores at follow-up and those at baseline; a negative change score indicated cognitive decline; †There were no any dementia events for ≥3 cerebellar CMBs and results were shown for overall cerebellar CMBs only;

‡ Model 1 was adjusted for age and sex

§ Model 2 was further adjusted for follow-up time interval, coil type, primary education, depression at follow-up, hypertension, total cholesterol, use of lipid-lowering medication, the presence of brain infarcts, measure of white matter hyperintensity volume expressed as percentage of total intracranial volume and Apolipoprotein E ε4 carriership.

Supplementary table 3. Association (β regression coefficient [95%confidence interval]) of prevalent cerebral microbleeds (CMBs) by location with 5-year cognitive change* (decline in Z scores) when additionally adjusting for the number of CMBs

	Memory		Processing speed		Working memory /executive function		Global cognitive function	
	Model 1 †	Model 2‡	Model 1†	Model 2‡	Model 1†	Model 2‡	Model 1†	Model 2‡
No CMBs (n=2129)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)
Overall strictly lobar CMBs (n=306)	0.05 (-0.11 to 0.21)	0.06 (-0.10 to 0.22)	0.09 (-0.05 to 0.24)	0.10 (-0.05 to 0.24)	-0.06 (-0.21 to 0.09)	-0.04 (-0.20 to 0.11)	0.01 (-0.15 to 0.17)	0.02 (-0.14 to 0.19)
Overall strictly deep CMBs (n=33)	-0.17 (-0.54 to 0.20)	-0.18 (-0.55 to 0.19)	0.006 (-0.34 to 0.35)	-0.002 (-0.35 to 0.34)	-0.50 (-0.87 to -0.12)	-0.50 (-0.88 to -0.13)	-0.26 (-0.64 to 0.12)	-0.27 (-0.65 to 0.11)
Overall mixed CMBs (n=25)	-0.60 (-1.10 to -0.09)	-0.56 (-1.07 to- 0.06)	-0.28 (-0.73 to 0.18)	-0.25 (-0.70 to 0.20)	-0.50 (-0.99 to -0.01)	-0.46 (-0.95 to 0.03)	-0.79 (-1.32 to -0.36)	-0.74 (-1.27 to- 0.21)

*Cognitive change was defined as the difference between composite cognitive z scores at follow-up and those at baseline; a negative change score indicated cognitive decline;

† Model 1 was adjusted for age, sex & CMBs count

‡ Model 2 was further adjusted for follow-up time interval, coil type, primary education, depression at follow-up, hypertension, total cholesterol, use of lipid-lowering medication, the presence of brain infarcts, measure of white matter hyperintensity volume expressed as percentage of total intracranial volume and Apolipoprotein E ε4 carriership.

Supplementary table 4. Association (β regression coefficient [95%confidence interval]) of prevalent cerebral microbleeds (CMBs) with 5-year cognitive decline when participants with baseline prevalent dementia were included (n=2,596)

	Memory		Processing speed		Working memory /executive function	
	Model 1†	Model 2 ‡	Model 1†	Model 2 ‡	Model 1†	Model 2 ‡
CMBs, by number						
No CMBs (n=2157)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)	0 (reference)
1 CMB (n=311)	0.10 (-0.03 to 0.22)	0.08 (-0.05 to 0.20)	0.03 (-0.08 to 0.15)	0.01 (-0.11 to 0.13)	-0.04 (-0.16 to 0.09)	-0.04 (-0.16 to 0.09)
2 CMBs (n=67)	-0.24 (-0.49 to 0.01)	-0.17 (-0.42 to 0.08)	-0.21 (-0.45 to 0.03)	-0.16 (-0.41 to 0.08)	-0.12 (-0.37 to 0.13)	-0.09 (-0.34 to 0.17)
≥3 CMBs (n=61)	-0.37 (-0.64 to -0.10)	-0.31 (-0.59 to -0.04)	-0.45 (-0.70 to -0.20)	-0.29 (-0.54 to -0.03)	-0.17 (-0.44 to 0.10)	-0.19 (-0.47 to 0.08)
P for trend	0.0369	0.097	0.003	0.037	0.122	0.135
Overall CMBs (n=439)	-0.02 (-0.13 to 0.09)	-0.02 (-0.13 to 0.10)	-0.06 (-0.17 to 0.03)	-0.06 (-0.16 to 0.05)	-0.07 (-0.17 to 0.04)	-0.06 (-0.17 to 0.05)

Supplementary table 4. Association (β regression coefficient [95%confidence interval]) of prevalent cerebral microbleeds (CMBs) with 5-year cognitive decline when participants with baseline prevalent dementia were included (n=2,596) (continued)

	Memory		Processing speed		Working memory /executive function	
CMBs, by location & number						
1 strictly lobar CMB (n=241)	0.10 (-0.04 to 0.24)	0.08 (-0.06 to 0.23)	0.03 (-0.11 to 0.16)	0.001 (-0.13 to 0.13)	0.01 (-0.13 to 0.15)	0.01 (-0.13 to 0.15)
2 strictly lobar CMBs (n=35)	-0.20 (-0.53 to 0.13)	-0.16 (-0.50 to 0.17)	-0.06 (-0.38 to 0.27)	-0.04 (-0.36 to 0.29)	-0.06 (-0.41 to 0.29)	-0.03 (-0.38 to 0.31)
≥3 strictly lobar CMBs (n=33)	-0.32 (-0.68 to 0.03)	-0.29 (-0.65 to 0.08)	-0.55 (-0.88 to -0.22)	-0.34 (-0.69 to 0.01)	-0.16 (-0.53 to 0.20)	-0.25 (-0.62 to 0.13)
P for trend	0.325	0.393	0.035	0.179	0.503	0.412
Overall strictly lobar CMBs (n=309)	0.01 (-0.11 to 0.14)	0.01 (-0.12 to 0.14)	-0.04 (-0.16 to 0.07)	-0.03 (-0.15 to 0.09)	-0.02 (-0.14 to 0.11)	-0.02 (-0.15 to 0.10)
1 deep and/or mixed CMB (n=25)	-0.11 (-0.52 to 0.30)	-0.18 (-0.60 to 0.24)	-0.20 (-0.58 to 0.19)	-0.28 (-0.67 to 0.12)	-0.46 (-0.89 to -0.04)	-0.50 (-0.94 to- 0.06)
2 deep and/or mixed CMBs(n=18)	-0.53 (-1.02 to -0.04)	-0.35 (-0.86 to 0.16)	-0.54 (-1.00 to -0.08)	-0.41 (-0.88 to 0.06)	-0.40 (-0.87 to 0.08)	-0.33 (-0.82 to 0.17)
≥3 deep and/or mixed CMBs(n=17)	-0.76 (-1.29 to -0.23)	-0.66 (-1.18 to -0.13)	-0.71 (-1.17 to -0.26)	-0.58 (-1.04 to -0.13)	-0.34 (-0.86 to 0.18)	-0.29 (-0.81 to 0.24)
P for trend	0.0005	0.0043	<.0001	0.0011	0.011	0.028
Overall deep or mixed CMBs (n=60)	-0.41 (-0.68 to -0.14)	-0.36 (-0.64 to- 0.08)	-0.44 (-0.70 to -0.19)	-0.40 (-0.66 to -0.14)	-0.41 (-0.69 to -0.14)	-0.39 (-0.67 to -0.11)

†Model 1 was adjusted for age and sex ‡Model 2 was further adjusted for follow-up time interval, coil type, primary education, depression at follow-up, hypertension, total cholesterol, use of lipid-lowering medication, the presence of brain infarcts, measure of white matter hyperintensity volume expressed as percentage of total intracranial volume and Apolipoprotein E ε4 carriership.

SUPPLEMENTARY REFERENCES

1. Saczynski JS, Sigurdsson S, Jonsdottir MK, et al. Cerebral infarcts and cognitive performance: importance of location and number of infarcts. *Stroke*. 2009; 40: 677-682.
2. Sigurdsson S, Aspelund T, Forsberg L, et al. Brain tissue volumes in the general population of the elderly: the AGES-Reykjavik study. *Neuroimage*. 2012; 59: 3862-3670.
3. van Sloten TT, Sigurdsson S, van Buchem MA, et al. Cerebral Small Vessel Disease and Association With Higher Incidence of Depressive Symptoms in a General Elderly Population: The AGES-Reykjavik Study. *Am J Psychiatry*. 2015;172:570-578.
4. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34: 939-944.
5. Chui HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. *Neurology*. 1992; 42: 473-480.
6. Lopez OL, Kuller LH, Becker JT, et al. Classification of vascular dementia in the Cardiovascular Health Study Cognition Study. *Neurology*. 2005; 64:1539-1547.
7. Ding J, Sigurdsson S, Garcia M, et al. Risk factors associated with incident cerebral microbleeds according to location in older people: The Age, Gene/Environment Susceptibility (AGES)-Reykjavik study. *JAMA Neurol*. 2015;72: 682-688.
8. Qiu C, Cotch MF, Sigurdsson S, et al. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology*. 2010;75: 2221-2228.