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Association of common genetic variants with brain microbleeds: A Genome-wide Association Study

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Appendix 2 - Coninvestigators - http://links.lww.com/WNL/B222

Abstract

Objective: To identify common genetic variants associated with the presence of brain microbleeds (BMB).

Methods: We performed genome-wide association studies in 11 population-based cohort studies and 3 case-control or case-only stroke cohorts. Genotypes were imputed to the Haplotype Reference Consortium or 1000 Genomes reference panel. BMB were rated on susceptibility-weighted or T2*-weighted gradient echo magnetic resonance imaging sequences, and further classified as lobar, or mixed (including strictly deep and infratentorial, possibly with lobar BMB). In a subset, we assessed the effects of *APOE* ε2 and ε4 alleles on BMB counts. We also related previously identified cerebral small vessel disease variants to BMB.

Results: BMB were detected in 3,556 of the 25,862 participants, of which 2,179 were strictly lobar and 1,293 mixed. One locus in the *APOE* region reached genome-wide significance for its association with BMB (lead SNP rs769449; $OR_{any\ BMB}$ (95% CI)=1.33 (1.21-1.45); $p=2.5x10^{-10}$). *APOE* ε4 alleles were associated with strictly lobar (OR (95% CI)=1.34 (1.19-1.50); $p=1.0x10^{-6}$) but not with mixed BMB counts (OR (95% CI)=1.04 (0.86-1.25); p=0.68). *APOE* ε2 alleles did not show associations with BMB counts. Variants previously related to deep intracerebral haemorrhage and lacunar stroke, and a risk score of cerebral white matter hyperintensity variants, were associated with BMB.

Conclusions: Genetic variants in the *APOE* region are associated with the presence of BMB, most likely due to the *APOE* ε4 allele count related to a higher number of strictly lobar BMB. Genetic predisposition to small vessel disease confers risk of BMB, indicating genetic overlap with other cerebral small vessel disease markers.

Introduction

Brain microbleeds (BMB), also referred to as cerebral microbleeds or cerebral microhemorrhages, correspond to hemosiderin deposits as a result of microscopic hemorrhages that are visible on magnetic resonance imaging (MRI) sequences.[1] The frequency of BMB increases with age and with certain pathologies including cerebral small vessel disease,[2] and in prospective studies BMB can predict risk of ischemic stroke and intracerebral hemorrhage (ICH).[3, 4] It has been suggested BMB may represent a marker that can stratify risk, particularly risk of ICH, in patients taking antithrombotic and anticoagulant therapy.[5]

Microbleeds can occur in both the cortical area or the cortico-subcortical border (lobar), and the subcortical (deep) structures of the brain. BMB in lobar regions are often seen in both familial and sporadic cerebral amyloid angiopathy, while deep BMB are more common in sporadic deep perforator arteriopathy.[6-8] This suggests that different pathophysiological mechanisms may underlie BMB in the two locations; a situation similar to that of ICH, where the genetic risk factor profiles for lobar and deep hemorrhage have been shown to differ.[9]

BMB represent one of a spectrum of MRI markers of cerebral small vessel disease, with others including white matter hyperintensities (WMH) and lacunar infarcts.[1] Genome-wide association studies (GWAS) of these other markers, particularly WMH, have provided novel insights into the underlying disease mechanisms.[10, 11] However much less is known of the genetic basis of BMB.[12, 13] We hypothesized that common genetic variants contribute to interindividual variation in BMB. Therefore, we performed the largest GWAS on BMB to date to evaluate this. In addition to any BMB, we performed separate GWAS for lobar BMB and mixed BMB.

Methods

Study population

The study included data from two large initiatives; the Cohorts of Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium[14] and the UK Biobank (http://www.ukbiobank.ac.uk), combined with additional data from the case-control Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu), and the MGH-GASROS[15] and CROMIS-2 AF[4] stroke studies. Together this comprised a total of 25,862 individuals from 9 population-based and 2 family-based cohort studies, as well as 1 case-control study and 2 case-only cohorts (**Table 1**).

Standard Protocol Approvals, Registrations, and Patient Consents

The individual studies have been approved by their local Institutional Review boards or ethic committees. Written informed consent was obtained from all individuals participating in the study.

Genotyping

Genotyping was performed on commercially available assays from Illumina or Affymetrix and were imputed using the Haplotype Reference Consortium or 1000 Genomes reference panels (**Supplementary Table 1** http://doi.org/10.5061/dryad.mcvdncjz4). Most cohorts included European ancestry individuals only, but a subset of Chinese, Malay and African American ancestry (N=130, N=204 and N=422, respectively) was also included.

Assessment of brain microbleeds

MRI scans with field strengths of 1T, 1.5T or 3T and full brain coverage were acquired in each participating study (**Supplementary Table 2** http://doi.org/10.5061/dryad.mcvdncjz4). Definitions of BMB have been described previously.[16] Briefly, BMB can be recognized as small, hypointense lesions on susceptibility weighted imaging (SWI) sequences or, to a lesser extent, on T2*-weighted gradient echo sequences. Although BMB assessment using SWI sequences is more sensitive than assessment using T2*-weighted sequences,[17, 18] the clinical relevance of this improved sensitivity is debated since it is also less specific.[19] Since previous research has shown differences between risk factors and clinical correlates of BMB in specific locations of the brain,[6, 8, 20] we further differentiated between strictly lobar, and deep, infratentorial or mixed BMB. Cases in which there were microbleeds located in cortical grey or subcortical white matter of the brain lobes without any microbleeds in deep or infratentorial regions were classified as lobar BMB. Microbleeds in the deep grey matter of basal ganglia and thalamus, or in brainstem or cerebellum were classified as deep or infratentorial BMB. Due to the low number of cases of BMB, especially the deep and infratentorial subtypes, we created one group of mixed BMB cases. Mixed BMB was defined as deep or infratentorial BMB, possibly in combination with microbleeds in lobar regions. In a minority of cohorts (see **Table 1**) the data on lobar and/or mixed BMB was not available, and therefore the total number of lobar and mixed BMB is slightly less than the total number of BMB. Study-specific methodologies for the identification of BMB have been described elsewhere.[1, 6, 21-30] Since the BMB assessment in the UK Biobank has not been described before, additional information regarding the UK Biobank sample, including microbleeds assessment, is provided in the **Supplementary Information** (http://doi.org/10.5061/dryad.mcvdncjz4).

Genome-wide association studies

In each participating study, genome-wide association analyses were performed using logistic regression under an additive model, adjusted for age, sex, and principal components of ancestry to account for population structure (if needed), and family relations (if applicable). For each study, variants were filtered by imputation quality (IQ) using an INFO or r^2 above 0.5, minor allele frequency (MAF) above 0.005 and MAF*N_{cases}*IQ>5. Within the CHARGE consortium plus additional case-control and case-only studies, only variants available in at least two cohorts were analyzed. Then, genetic variants were filtered using MAF>0.01, after which the CHARGE consortium with additional studies and UK Biobank results were meta-analyzed together. An inverse-variance weighted fixed-effects model was applied in METAL using the standard error analysis scheme.[31] As a sensitivity analysis, we performed this analysis while excluding individuals with dementia and stroke, to investigate whether the associations were driven by these diseases. To examine whether there was substantial genomic inflation due to population stratification, we inspected the LD score regression intercept (**Supplementary Table 3** http://doi.org/10.5061/dryad.mcvdncjz4).[32] For follow-up analyses, only variants present in more than half of the cases were included. HaploReg v4.1 was used for the functional annotation of the suggestive ($p<5x10^{-6}$) and genome-wide significant ($p<5x10^{-8}$) variants, and variants in linkage disequilibrium (LD) at a threshold of $r^2>0.8$.[33]

APOE ε2 and ε4 count analysis

In the two largest cohorts (i.e. UK Biobank and Rotterdam Study), we investigated the effect of *APOE* ε2 and ε4 allele counts, directly genotyped using a polymerase chain reaction, inferred from imputed Haplotype Reference Consortium values of rs429358 and rs7412, or a combination of both. Zero-inflated negative binomial regression analysis was performed

investigating the association of *APOE* allele counts with the number of any, lobar and mixed BMB, adjusted for age, sex and principal components. For each individual, we counted the number of *APOE* ε2 alleles (ε2ε2 coded as 2, ε2ε3 and ε2ε4 as 1, and ε3ε3, ε3ε4 and ε4ε4 as 0), and the number of *APOE* ε4 alleles (ε4ε4 coded as 2, ε2ε4 and ε3ε4 as 1, and ε2ε2, ε2ε3 and ε3ε34 as 0). We repeated these analyses while setting *APOE* ε2ε4 values to missing since this combines the protective ε2 and the risk-increasing ε4 allele for Alzheimer's disease and may therefore dilute the effects. For these analyses, counts of more than 100 microbleeds were considered outliers and removed from the analysis (N=2 in the UK Biobank; N=2 in the Rotterdam Study).

Two-sample Mendelian randomization

In order to test potential causal effects of cardiovascular risk factors on BMB, we performed a two-sample Mendelian randomization using an inverse-variance weighted method implemented in the MendelianRandomization R library. Summary statistic data of GWAS were acquired for the following traits: type 2 diabetes mellitus,[34] systolic and diastolic blood pressure, pulse pressure,[35] body mass index,[36] low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides[37].

Related phenotypes

For independent ($r^2 \leq 0.8$) variants previously associated at genome-wide significance with other traits which in turn might be related to BMB, we assessed the association with BMB as well. First we examined variants associated with other manifestations of cerebral small vessel disease, namely WMH,[10, 11, 15] lacunar stroke,[38, 39] and ICH[39, 40]. Second we examined associations with traits which have been shown to be predicted by BMB, namely any stroke, any ischemic stroke,[41, 42] and Alzheimer's disease[43]. For each related phenotype, we corrected the p-value for significance, dividing 0.05 by the number of single nucleotide polymorphisms

(SNPs) tested. Where we had a sufficient number of variants we assessed the cumulative association of all variants with BMB using inverse variance weighting across all SNPs, as implemented in the gtx package in R. For WMH the effect sizes from the largest GWAS sample were used to estimate an overall effect.[10]

Data availability statement

The summary statistics will be made available upon publication on the CHARGE dbGaP site under the accession number phs000930.v7.p1.

Results

In the combined CHARGE with additional studies and UK Biobank multi-ethnic meta-analysis, genetic and BMB rating data were available for 25,862 subjects, of whom 3,556 (13.7%) had BMB. In 2,179 (8.4%) these were lobar and in 1,293 (5.0%) mixed. The prevalence of any BMB ranged from 6.5% to 34.3% for studies using T2*-weighted sequences for the assessment of BMB, and from 7.0% to 36.8% for studies using SWI sequences. After excluding subjects with dementia and stroke 23,032 individuals remained, of whom 2,889 (12.5%), 1,843 (8.0%) and 969 (4.2%) had any, lobar, and mixed BMB, respectively. A complete overview of the included studies is shown in **Table 1**.

Genome-wide association studies

A quantile-quantile plot showed mild enrichment of genome-wide associations with any BMB (**Supplementary Figure 1** http://doi.org/10.5061/dryad.mcvdncjz4), and limited genomic inflation was observed (λ=1.02, LD score regression intercept=1.02, **Supplementary Table 3** http://doi.org/10.5061/dryad.mcvdncjz4). One locus in the *APOE* region on chromosome 19 reached genome-wide significance (lead genetic variant rs769449; OR (95% CI)=1.33 (1.21-1.45); p=$2.5x10^{-10}$; **Table 2**, **Figure 1-2**, **Supplementary Figure 2** http://doi.org/10.5061/dryad.mcvdncjz4). This effect was stronger for lobar (OR (95% CI)=1.32 (1.19-1.47); p=$4.3x10^{-7}$) than for mixed microbleeds (OR (95% CI)=1.27 (1.11-1.46); p=$5.4x10^{-4}$), albeit not significantly. Similar associations were observed for the different participating studies (CHARGE with additional studies I^2=0, $p_{heterozygosity}$=0.68; CHARGE with additional studies and UK Biobank combined I^2=0, $p_{heterozygosity}$=0.78, **Supplementary Figure 3** http://doi.org/10.5061/dryad.mcvdncjz4). Functional annotation of the genome-wide significant variants and genetic variants in LD (r^2>0.8) are presented in

Supplementary Table 4 http://doi.org/10.5061/dryad.mcvdncjz4). In the analysis excluding individuals with dementia and stroke, the effect estimate for the lead SNP rs769449 did not attenuate, although the level of significance slightly decreased reflecting the smaller sample size (OR (95% CI)=1.32 (1.20-1.46), p=2.1x10^{-8}, **Supplementary Table 5**, **Supplementary Figure 4** http://doi.org/10.5061/dryad.mcvdncjz4).

APOE ε2 and ε4 count analysis

To further elucidate whether one of the two *APOE* genotypes were driving this identified genetic association between the *APOE* region and BMB, we performed a follow-up analysis of this finding, assessing the association of *APOE* ε2 and ε4 allele counts with BMB in the two largest cohorts (Rotterdam Study and UK Biobank). The *APOE* ε4 allele count was significantly associated with the number of BMB (OR (95% CI)=1.27 (1.14-1.42); p=1.3 x 10^{-5}, **Table 3**). This effect was stronger for lobar than for mixed microbleeds (OR (95% CI)=1.33 (1.16-1.52); p=3.5 x 10^{-5} and OR (95% CI)=1.07 (0.85-1.35); p=0.553, respectively). These results did not change after excluding individuals with the *APOE* ε2ε4 genotype (**Supplementary Table 6** http://doi.org/10.5061/dryad.mcvdncjz4). No significant association was found between the *APOE* ε2 allele count and the number of BMB (OR (95% CI)=1.03 (0.86-1.22); p=0.769), also not after removing individuals with the *APOE* ε2ε4 genotype (**Table 3**, **Supplementary Table 6** http://doi.org/10.5061/dryad.mcvdncjz4).

Two-sample Mendelian randomization

Mendelian randomization analyses testing the influence of cardiovascular risk factors on BMB showed positive nominal associations of systolic blood pressure, diastolic blood pressure, and triglycerides with any BMB; and of systolic and diastolic blood pressure and triglycerides with strictly lobar BMB; as well as triglycerides with deep, infratentorial or mixed BMB (**Table 4**). Only the association of triglycerides with any microbleeds survived

multiple testing adjustments (β=0.29, 95% CI=0.09-0.49, p=0.004); the effect estimate of this association was stronger for mixed microbleeds (β=0.37, 95% CI=0.09-0.65, p=0.009).

Related phenotypes

One genetic variant previously associated with deep ICH and WMH (rs2984613 in the 1q22 locus) was associated with BMB (OR (95% CI)=1.12 (1.05-1.18), p=1.8 x 10^{-4}), with slightly stronger effects on mixed BMB than lobar BMB (OR (95% CI)=1.14 (1.05-1.25), p=3.2 x 10^{-3} versus OR (95% CI)=1.09 (1.01-1.17), p=2.2 x 10^{-2}) (**Table 5**). One variant known to be associated with lacunar stroke (rs9515201 in the 13q34 locus) also associated with mixed BMB (OR (95% CI)=1.12 (1.02-1.22), p=0.014), but did not associate with lobar BMB (OR (95% CI)=0.98 (0.91-1.06), p=0.684). No other cerebral small vessel disease variants were individually associated with BMB. Yet cumulatively, genetic variants identified for cerebral WMH burden were associated with mixed BMB (OR (95% CI)=1.78 (1.15-2.77); p=0.01), but not with lobar BMB (OR (95% CI)=1.02 (0.71-1.45); p=0.93). Also, a cumulative effect of previously identified variants for any stroke was found for mixed BMB (OR (95% CI)=1.78 (1.09-2.91); p=0.02), which was similar for variants of any ischemic stroke (OR (95% CI)=2.00 (1.22-3.27); p=0.006). Full results of the genetic variants previously identified for Alzheimer's disease and stroke are presented in **Supplementary Table 7** (http://doi.org/10.5061/dryad.mcvdncjz4).

Discussion

We report the first large-scale multi-ethnic genome-wide study of BMB in 25,862 individuals, including 3,556 subjects with any BMB of whom 2,179 had strictly lobar and 1,293 mixed BMB. We identified an association with BMB in the *APOE* region, in particular for strictly lobar BMB, most likely due to risk associated with *APOE* ε4 allele counts.

Our findings are in line with previous studies showing an association between *APOE* ε4 genotypes and BMB, in particular with strictly lobar BMB.[12] One genetic variant in LD with the identified lead SNP (rs769448) is rs429358, which is an *APOE* missense variant and one of the two SNPs constituting *APOE* ε2/3/4 polymorphisms; this variant was more strongly associated with strictly lobar than mixed BMB. In an additional analysis performed in a subset of the cohorts we confirmed the known link between *APOE* ε4 allele count and the number of BMB, with stronger effect estimates for the strictly lobar BMB subtype compared to the mixed subtype. This association was less pronounced and non-significant for the *APOE* ε2 allele count, which is also in accordance with previous studies,[12] although this might still be due to a lack of power. Other studies did find a significant association between *APOE* ε2 alleles and cerebral angiopathy related ICH,[9] with stronger estimates for the lobar compared to the deep phenotype, which is similar to our study. Stronger effects for ICH in the previous study than for BMB in the current study might be due to sampling variability or biological differences between the two traits. The *APOE* locus remained significant with a similar effect estimate in the GWAS meta-analysis performed in a dementia- and stroke-free sample, indicating that this association was not driven by diseased individuals, and suggesting that *APOE* may already affect BMB risk in a preclinical phase of dementia or stroke.

Our findings further suggest that higher triglyceride levels may be causally related to the presence of BMB. This relationship between the genetics of triglycerides and BMB, in particular for mixed BMB, confirms other studies showing a contribution of cardiovascular risk factors to BMB risk, mainly for deep or infratentorial BMB.[6] A previous two-sample Mendelian randomization study did not find a significant association between the genetics of triglycerides and ICH, although the direction of effect for the triglycerides analysis was the same as for BMB in the current study.[44] However, this positive link between the genetics of triglyceride levels and the presence of BMB is in contrast with previous phenotypic association studies showing an inverse relationship between triglyceride levels and BMB risk in elderly population-based individuals.[45, 46] Similarly, lower triglyceride levels have been associated with an increased ICH risk.[45, 47, 48] Thus, our finding should be interpreted with caution and further studies are needed to elucidate the exact causal mechanisms underlying lipid profiles over time and BMB risk.

We also showed that genetic variation previously associated with risk of cerebral small vessel disease (i.e. WMH burden, lacunar infarcts and subcortical ICH) are associated with an increased risk of BMB, and that this association is restricted to mixed rather than lobar BMB. This suggests that mixed BMB have a shared pathophysiological pathway with other features of the cerebral small vessel disease spectrum. This is consistent with recent data showing genetic sharing between WMH, lacunar infarcts and subcortical ICH.[49] Additionally, increasing evidence suggests that small vessel arteriopathy may lead to WMH, acute lacunar infarction and ICH.[50] Our data suggests that mixed BMB are likely to be related to the same underlying arterial pathology.

Associations of the *APOE* ε4 genotype with decreased cognitive function in the elderly are well established.[51] Although part of this decline is due to the predisposition to Alzheimer's disease pathology conferred by *APOE* ε4, our results suggest that another part might be due to

vascular mechanisms predisposing to BMB, most likely via cerebral amyloid angiopathy. Apart from the *APOE* locus, no enrichment of previously reported genetic variants for Alzheimer's disease was found. This is in line with a previously published WMH GWAS, in which no significant association was found between the identified loci for WMH and Alzheimer's disease.[11] It might indicate that *APOE* is mainly responsible for the genetic overlap between BMB and Alzheimer's disease. Alternatively, the current BMB and Alzheimer's disease GWAS could still be underpowered to identify biological pathways playing a role in the development of cerebral small vessel disease subsequently leading to Alzheimer's disease. As another possibility, environmental factors might primarily play a role in the link between BMB and neurodegenerative diseases later in life. Although the 19q13 locus was the only significant BMB locus, we did observe a cumulative effect of stroke SNPs on mixed BMB, suggestive of overlapping biological mechanisms underlying the two.

In this study, we were able to collate most of the GWAS data available worldwide on BMB, enabling us to perform by far the largest GWAS meta-analysis of BMB to date. However, our study also has limitations. Despite being the largest study to date, the number of individuals with BMB was still modest, resulting in a limited power to identify genetic factors related to BMB. Significantly larger sample sizes are needed to fully elucidate the genetic contribution to BMB. Because of the relatively small number of participants with BMB we combined the presence of deep, infratentorial and mixed BMB into one group of "mixed" BMB, even though previous research has suggested there may be differences between differences between strictly deep and mixed BMB.[20] With larger sample sizes it would be interesting to investigate whether there are differences in the genetics between deep and infratentorial BMB. The percentage of individuals with microbleeds varied across studies, which may be due to a true difference in the presence of BMB, due to population differences, e.g. age distributions, ethnicities and life style factors. However, the differences in the presence of BMB might also

be partially attributable to different sensitivities of the used methodologies, e.g. the magnetic field strength of the MRI scanner or the sequence used for rating BMB. In addition, a limitation of the current study is the large majority of European ancestry individuals included in the analyses, although previous studies have shown differences in the occurrence, distribution and associated risks of BMB across different ethnicities.[52-54] Therefore, it would be valuable for future studies to increase the sample size of non-European ancestry individuals in order to be able to perform ancestry-specific analyses. Also, larger reference panels would enable us to investigate rare genetic variants as well. Lastly, it may be worthwhile to take into account the number of microbleeds instead of treating the phenotype as a dichotomous trait, which results in a loss of information.

In conclusion, we identified genetic variants located in the *APOE* region associated with BMB, which were more strongly associated with lobar than mixed BMB. Our data also demonstrated genetic overlap between mixed BMB, and other features of cerebral small vessel disease, emphasizing that they represent part of the cerebral small vessel disease spectrum.

Tables and figures

Table legends

Table 1. Population characteristics of contributing studies.

Table 2. Independent genetic variants significantly ($p<5x10^{-8}$) or suggestively ($p<1x10^{-6}$) associated with any or location-specific brain microbleeds.

Table 3. The effects of *APOE* ε4 allele count on the number of brain microbleeds overall and by location.

Table 4. Two-sample Mendelian randomization of cardiovascular traits and brain microbleeds overall and by location.

Table 5. Association of subcortical small vessel disease associated genetic variants with brain microbleeds overall and by location.

Figure legends

Figure 1. Common genetic variants associated with brain microbleeds. Manhattan plots showing genome-wide associations by chromosomal position for (A) any, (B) lobar and (C) mixed microbleeds.

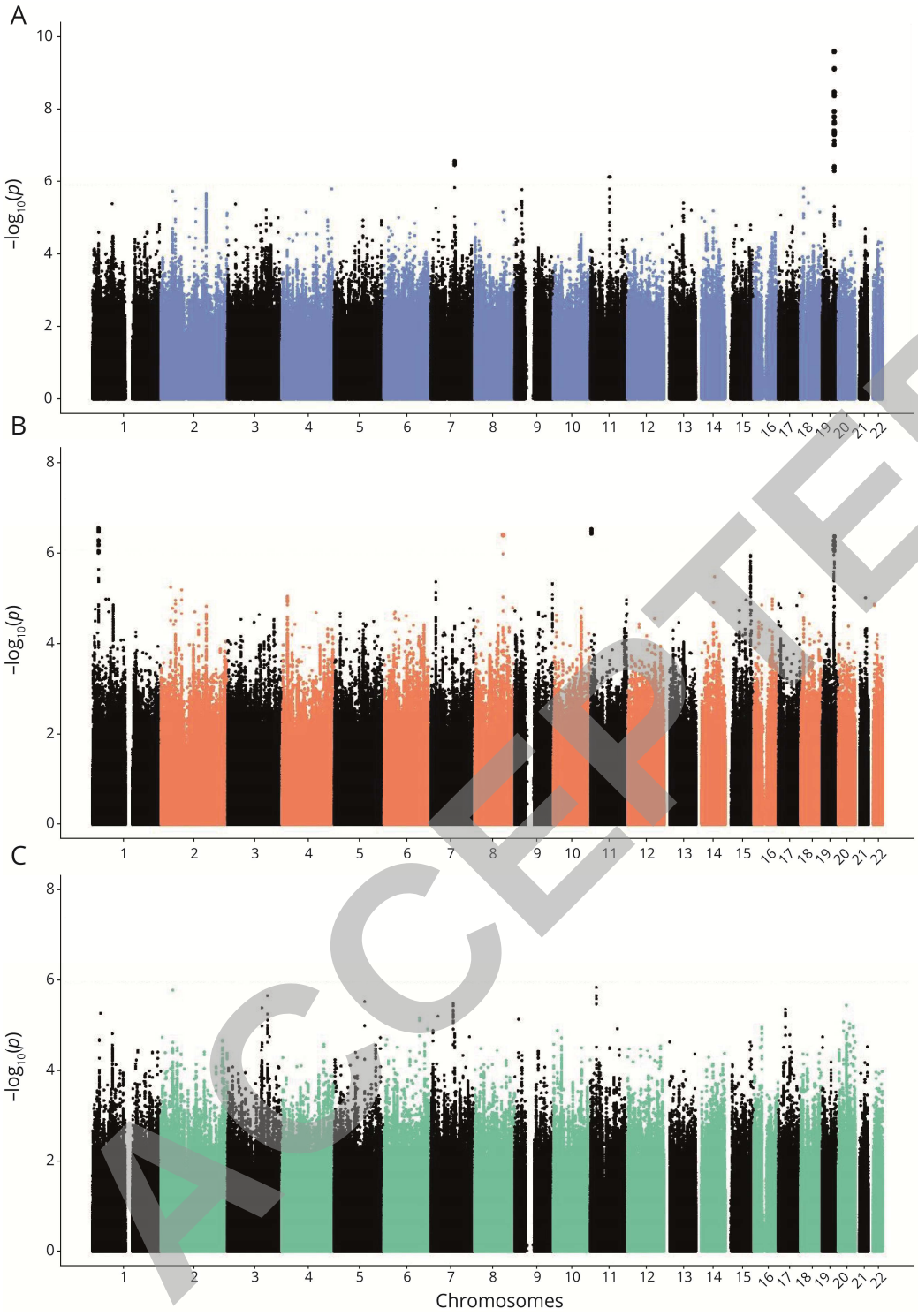
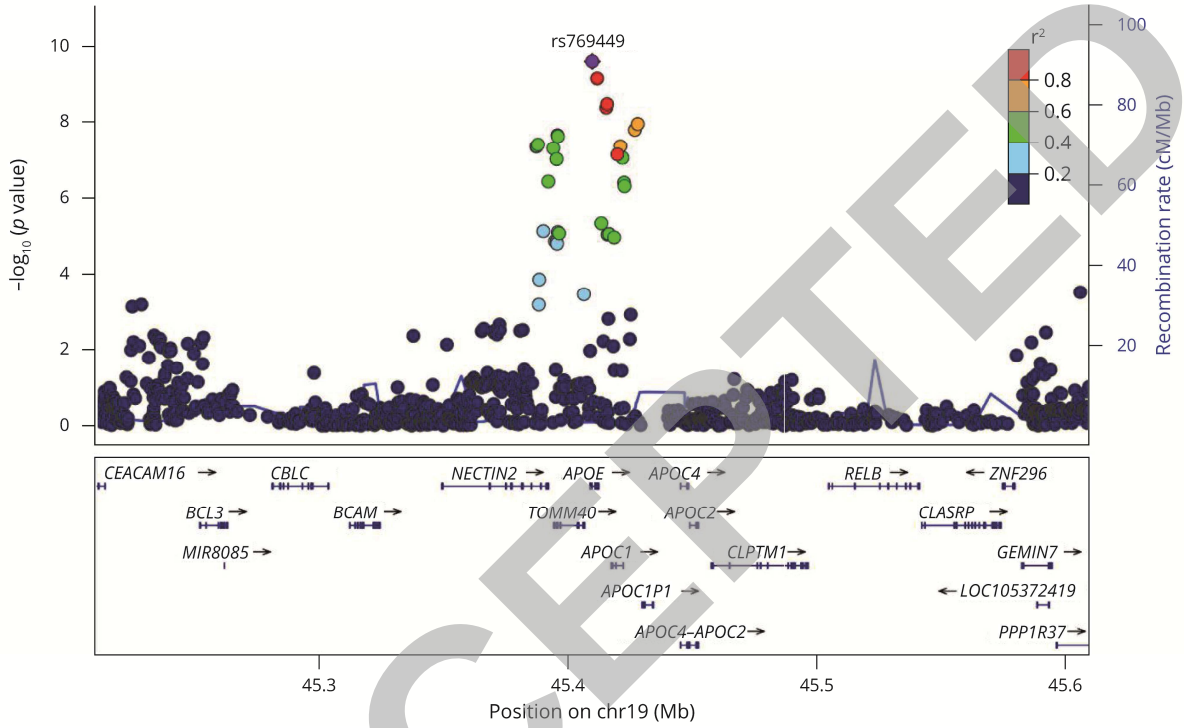


Figure 2. Regional association of genome-wide significant locus for any brain microbleeds. Regional plot showing association of genetic variants in the *APOE* region with any brain microbleeds.



Supplementary Table legends

Supplementary Table 1. (http://doi.org/10.5061/dryad.mcvdncjz4) Genotyping and quality control metrics.

Supplementary Table 2. (http://doi.org/10.5061/dryad.mcvdncjz4) Image data acquisition and processing.

Supplementary Table 3. (http://doi.org/10.5061/dryad.mcvdncjz4) Genomic inflation and polygenicity in the different meta-analyses.

Supplementary Table 4. (http://doi.org/10.5061/dryad.mcvdncjz4) Functional annotation of genome-wide significant and suggestive genetic variants for brain microbleeds ($p<1x10^{-6}$) and variants in linkage disequilibrium ($r^2>0.8$).

Supplementary Table 5. (http://doi.org/10.5061/dryad.mcvdncjz4) Independent genome-wide significant and suggestive associations ($p<1x10^{-6}$) with brain microbleeds in a sample excluding individuals with dementia or stroke.

Supplementary Table 6. (http://doi.org/10.5061/dryad.mcvdncjz4) The effects of *APOE* ε4 allele count on the number of brain microbleeds overall and by location, excluding individuals with the *APOE* ε2ε4 genotype.

Supplementary Table 7. (http://doi.org/10.5061/dryad.mcvdncjz4) Association of genetic variants for Alzheimer's disease and stroke with brain microbleeds overall and by location.

Supplementary Figure legends

Supplementary Figure 1. (http://doi.org/10.5061/dryad.mcvdncjz4) Quantile-quantile plots showing the observed versus expected –log P-value for (A) any, (B) lobar and (C) mixed microbleeds.

Supplementary Figure 2. (http://doi.org/10.5061/dryad.mcvdncjz4) Regional plots of the suggestive genetic variants ($p<1x10^{-6}$) for overall or location-specific microbleeds.

Supplementary Figure 3. Forest plots showing the study-specific associations between the independent genome-wide significant ($p<5x10^{-8}$) and suggestive ($p<1x10^{-6}$) genetic variants and brain microbleeds, overall and by location.

Supplementary Figure 4. Quantile-quantile plots and Manhattan plots presenting the results of the genome-wide association studies of (A) any, (B) lobar, and (C) mixed microbleeds in a study sample without dementia or stroke.

Study	Study design	Ancestry	N total	N any BMB	N lobar BMB	N mixed BMB	N (%) female	Age (mean±sd)	Age range	N demented	N stroke
ADNI	Case-control (AD, MCI, healthy controls)	European	734	149	95	54	330 (45.0)	73.1 ± 7.5	48 - 94	116	45
AGES	Population-based	European	2,894	469	272	197	1,679 (58.0)	76.4 ± 5.5	66 - 95	149	223
ASPS	Population-based	European	203	34	NA	28	89 (43.8)	60.1 ± 6.3	46 - 79	0	0
ARIC (AA)	Population-based	European	422	118	81	31	281 (66.6)	75.4 ± 5.1	67 - 89	24	22
ARIC (EA)	Population-based	African American	1,174	267	184	74	680 (57.9)	77.0 ± 5.3	67 - 90	70	34
CROMIS-2 AF	Case-only (stroke cases)	European	1,238	253	94	158	522 (42.2)	75.1 ± 12.6	35 - 100	32	1,238
EDIS-SCES	Population-based	Chinese	130	42	27	NA	69 (53.1)	70.5 ± 6.1	60 - 85	5	6
EDIS-SiMES	Population-based	Malay	204	75	36	NA	107 (52.5)	70.6 ± 6.6	60 - 85	21	8
ERF	Family-based	European	126	27	15	12	66 (52.4)	64.5 ± 4.6	55 - 75	0	0
FHS	Population-based	European	3,968	257	176	81	2,115 (53.3)	57.3 ± 13.6	25 - 96	25	51
LBC1936	Population-based	European	626	74	21	53	295 (47.1)	72.7 ± 0.7	71 - 74	5	43

LLS	Family-based	European	279	39	24	11	147 (52.7)	65.8 ± 6.9	45 - 84	0	0
MGH-GASROS	Case-only (stroke cases)	European	380	106	51	55	127 (36.0)	66.7 ± 15.0	18 - 102	0	353
PROSPER	RCT/population-based	European	456	104	74	26	197 (43.2)	75.0 ± 3.2	70 - 83	0	74
RS1	Population-based	European	1,119	384	234	150	642 (57.4)	79.2 ± 5.0	68 - 96	30	64
RS2	Population-based	European	1,206	270	167	103	628 (52.1)	69.7 ± 6.2	60 - 97	8	23
RS3	Population-based	European	2,611	318	237	81	1,444 (55.3)	57.3 ± 6.6	45 - 89	0	3
UK Biobank	Population-based	European	8,092	570	391	179	4,263 (52.7)	62.1 ± 7.4	44 - 78	3	75
			25,862	**3,556**	**2,179**	**1,293**					

Abbreviations: AA, African ancestry; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; AGES, Age Gene/Environment Susceptibility; ASPS, Austrian Stroke Prevention Study; ARIC, Atherosclerosis Risk in Communities; BMB, brain microbleeds; CROMIS-2 AF, Clinical Relevance of Microbleeds In Stroke due to Atrial Fibrillation; EA, European ancestry; EDIS, Epidemiology of Dementia in Singapore; ERF, Erasmus Rucphen Family; FHS, Framingham Heart Study; LBC1936, Lothian Birth Cohort 1936; LLS, Leiden Longevity Study; MCI, mild cognitive impairment; MGH-GASROS, Massachusetts General Hospital Genes Affecting Stroke Risk and Outcomes Study; N, number of subjects; PROSPER, PROspective Study of Pravastatin in the Elderly at Risk; RCT, randomized controlled trial; RS, Rotterdam Study; SCES, Singapore Chinese Eye Study; sd, standard deviation; SiMES, Singapore Malay Eye Study.

Table 1. Population characteristics of contributing studies.

Table 2. Independent genetic variants significantly ($p<5x10^{-8}$) or suggestively ($p<1x10^{-6}$) associated with any or location-specific brain microbleeds.

SNP	Chr	Position	A1	A2	EAF	Nearest gene	Outcome	Beta	SE	OR	N total	N cases	P-value
rs769449	19	45410002	A	G	0.13	*APOE*	Any BMB	0.282	0.045	1.33	20,150	2,858	2.5×10^{-10}
							Lobar BMB	0.280	0.055	1.32	18,666	1,748	4.3×10^{-7}
							Mixed BMB	0.243	0.070	1.27	18,319	1,049	5.4×10^{-4}
rs6950978	7	87200467	A	T	0.70	*ABCB1*	Any BMB	-0.154	0.030	0.86	25,528	3,439	2.7×10^{-7}
							Lobar BMB	-0.153	0.037	0.86	24,101	2,101	4.1×10^{-5}
							Mixed BMB	-0.179	0.046	0.84	23,033	1,239	1.0×10^{-4}
rs7533718	1	22281393	A	G	0.83	*HSPG2*	Any BMB	-0.140	0.042	0.87	25,402	3,412	7.5×10^{-4}
							Lobar BMB	-0.263	0.051	0.77	22,935	2,005	2.9×10^{-7}
							Mixed BMB	0.003	0.070	1.00	22,446	1,161	9.7×10^{-1}
rs11025317	11	3103445	A	G	0.12	*OSBPL5*	Any BMB	0.172	0.049	1.19	20,330	2,918	4.3×10^{-4}
							Lobar BMB	0.305	0.060	1.36	18,666	1,748	3.0×10^{-7}
							Mixed BMB	-0.027	0.082	0.97	17,714	996	7.4×10^{-1}
rs62522567	8	103799094	A	G	0.92	*GASAL1*	Any BMB	-0.231	0.051	0.79	24,118	3,115	6.9×10^{-6}
							Lobar BMB	-0.319	0.063	0.73	22,550	1,924	4.0×10^{-7}
							Mixed BMB	-0.195	0.089	0.82	17,075	942	2.8×10^{-2}

rs1058285	19	43680051	T	C	0.61	*PSG5*	Any BMB	0.082	0.030	1.08	24,794	3,290	6.0×10^{-3}
							Lobar BMB	0.188	0.038	1.21	23,535	2,021	5.3×10^{-7}
							Mixed BMB	-0.051	0.045	0.95	22,729	1,216	2.6×10^{-1}
rs654240	11	69448373	T	C	0.41	*CCND1*	Any BMB	0.154	0.031	1.17	25,402	3,412	7.4×10^{-7}
							Lobar BMB	0.116	0.039	1.12	23,528	2,080	2.8×10^{-3}
							Mixed BMB	0.202	0.048	1.22	23,368	1,270	3.0×10^{-5}

Table presenting the associations with brain microbleeds with a p<1x10-6. If available, the associations of the same genetic variants in the other analyses are also shown. *Abbreviations: A1, effect allele; A2, other allele; BMB, brain microbleeds; Chr, chromosome; EAF, effect allele frequency; N, number of subjects; OR, odds ratio; P, p-value; SE, standard error; SNP, single nucleotide polymorphism.*

Table 3. The effects of *APOE* ε2 and 4 allele count on the number of brain microbleeds overall and by location.

Outcome	Beta	SE	OR (95% CI)	P-value
***APOE* ε2 allele count**				
All BMB	0.026	0.089	1.03 (0.86-1.22)	0.769
Lobar BMB	0.130	0.121	1.14 (0.90-1.44)	0.283
Mixed BMB	-0.243	0.178	0.78 (0.55-1.11)	0.171
***APOE* ε4 allele count**				
All BMB	0.242	0.055	1.27 (1.14-1.42)	1.3×10^{-5}
Lobar BMB	0.285	0.069	1.33 (1.16-1.52)	3.5×10^{-5}
Mixed BMB	0.069	0.117	1.07 (0.85-1.35)	0.553

Abbreviations: BMB, brain microbleeds; CI, confidence interval; OR, odds ratio; SE, standard error.

Table 4. Two-sample Mendelian randomization of cardiovascular traits and brain microbleeds overall and by location.

Analysis	Estimate (95% CI)	P-value
Any brain microbleeds		
Type 2 diabetes	-0.072 (-0.176-0.031)	0.170
Systolic blood pressure	0.026 (0.005-0.046)	0.013*
Diastolic blood pressure	0.046 (0.010-0.082)	0.011*
Pulse pressure	0.021 (-0.008-0.049)	0.156
Body mass index	-0.037 (-0.131-0.057)	0.445
Low density lipoprotein	0.057 (-0.085-0.198)	0.431
High density lipoprotein	-0.001 (-0.159-0.157)	0.990
Triglycerides	0.290 (0.090-0.489)	0.004**
Lobar brain microbleeds		
Type 2 diabetes	-0.053 (-0.180-0.074)	0.414
Systolic blood pressure	0.027 (0.003-0.051)	0.029*
Diastolic blood pressure	0.046 (0.003-0.088)	0.035*
Pulse pressure	0.023 (-0.010-0.057)	0.174
Body mass index	-0.023 (-0.141-0.094)	0.697
Low density lipoprotein	0.145 (-0.015-0.306)	0.076
High density lipoprotein	-0.024 (-0.206-0.159)	0.799
Triglycerides	0.250 (0.015-0.486)	0.037*
Mixed brain microbleeds		

Type 2 diabetes	-0.074 (-0.222-0.073)	0.323
Systolic blood pressure	0.024 (-0.005-0.054)	0.108
Diastolic blood pressure	0.034 (-0.019-0.086)	0.209
Pulse pressure	0.025 (-0.017-0.066)	0.243
Body mass index	-0.047 (-0.191-0.097)	0.524
Low density lipoprotein	-0.078 (-0.315-0.159)	0.519
High density lipoprotein	-0.050 (-0.263-0.162)	0.642
Triglycerides	0.374 (0.094-0.654)	0.009*

Nominally significant associations are denoted by an asterisk (*; $p<0.05$), associations significant after adjustment for the number of risk factors by two asterisks (**; $p<(0.05/8)$).

Abbreviations: CI, confidence interval.

Table 5. Association of cerebral small vessel disease associated genetic variants with brain microbleeds overall and by location.

			All BMB		Lobar BMB		Mixed BMB	
Trait	**Locus**	**SNP**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
ICH deep	1q22	rs2984613	1.12 (1.05-1.18)	0.0002**	1.09 (1.01-1.17)	0.022**	1.14 (1.05-1.25)	0.003**
	13q34	rs4771674	1.03 (0.97-1.09)	0.350	0.99 (0.93-1.07)	0.879	1.06 (0.97-1.15)	0.218
Lacunar stroke	16q24	rs12445022	1.07 (1.00-1.13)	0.034*	1.04 (0.97-1.12)	0.277	1.10 (1.00-1.20)	0.039*
	10q26	rs79043147	1.02 (0.91-1.14)	0.785	1.04 (0.90-1.21)	0.601	1.05 (0.87-1.27)	0.582
	13q34	rs9515201	1.04 (0.98-1.10)	0.206	0.98 (0.91-1.06)	0.684	1.12 (1.02-1.22)	0.014**
WMH	2p21	rs11679640	0.95 (0.88-1.01)	0.111	0.96 (0.88-1.04)	0.300	0.98 (0.88-1.10)	0.768
	10q24	rs12357919	1.01 (0.94-1.08)	0.881	1.00 (0.91-1.10)	0.970	0.97 (0.86-1.09)	0.598
	6q25	rs275350	1.01 (0.95-1.06)	0.775	0.98 (0.91-1.05)	0.519	1.08 (0.99-1.17)	0.084
	1q22	rs2984613	1.12 (1.05-1.18)	0.0002**	1.09 (1.01-1.17)	0.022*	1.14 (1.05-1.25)	0.003**
	17q25	rs7214628	1.00 (0.94-1.08)	0.902	1.04 (0.95-1.13)	0.404	1.02 (0.91-1.13)	0.779
	10q24	rs72848980	1.00 (0.93-1.08)	0.947	1.00 (0.91-1.10)	0.970	0.98 (0.87-1.10)	0.687
	2q33	rs72934505	1.05 (0.96-1.15)	0.264	1.01 (0.91-1.12)	0.886	1.11 (0.97-1.27)	0.141
	2p16	rs78857879	1.06 (0.97-1.17)	0.206	1.02 (0.91-1.16)	0.695	1.08 (0.93-1.25)	0.300
	10q24	rs7894407	1.04 (0.98-1.10)	0.212	1.01 (0.94-1.09)	0.772	1.02 (0.94-1.12)	0.605
	10q24	rs7909791	0.99 (0.94-1.05)	0.784	0.99 (0.92-1.06)	0.737	0.96 (0.88-1.05)	0.420
	14q32	rs941898	0.95 (0.89-1.01)	0.117	0.91 (0.84-0.99)	0.026*	1.01 (0.92-1.12)	0.817
	13q34	rs9515201	1.04 (0.98-1.10)	0.206	0.98 (0.91-1.06)	0.684	1.12 (1.02-1.22)	0.014*
	17q21	rs962888	1.02 (0.96-1.08)	0.570	1.01 (0.93-1.09)	0.868	1.02 (0.93-1.12)	0.641
		Overall	1.29 (0.97-1.72)	0.074	1.02 (0.71-1.45)	0.927	1.78 (1.15-2.77)	0.010*

Odds ratios aligned to risk allele from original studies. Significance levels are denoted by asterisks: *, nominally significant ($p<0.05$); **, significant after Bonferroni correction ($p<0.05$/number of genetic variants). † In the overall score for WMH rs12357919 was left out since this genetic variant was in linkage disequilibrium ($r^2>0.2$) with rs72848980. *Abbreviations: BMB, brain microbleeds; CI,*

confidence interval; ICH, intracerebral haemorrhage; OR, odds ratio; SNP, single nucleotide polymorphism; WMH, white matter hyperintensities.

Appendix 1 – Authors

Name	Location	Contribution
Maria J Knol	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Performed statistical analysis; drafted the manuscript.
Dongwei Lu	University of Cambridge, Cambridge, United Kingdom	Acquired data.
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Lukas Pirpamer	Medical University of Graz, Graz, Austria	Acquired data.
Kejal Kantarci	Mayo Clinic, Rochester, United States of America	Acquired data.
Jayandra J Himali	Boston University, Boston, United States of America	Performed statistical analysis.
Qiong Yang	Boston University, Boston, United States of America	Acquired data.

Zoe Morris	University of Edinburgh, Edinburgh, United Kingdom	Acquired data.
Alexa S Beiser	Boston University, Boston, United States of America	Acquired data.
Daniel J Tozer	University of Cambridge, Cambridge, United Kingdom	Acquired data.
Meike W Vernooij	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data.
Najaf Amin	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data.
Marian Beekman	Leiden University Medical Center, Leiden, the Netherlands	Acquired data.
Jia Yu Koh	Singapore Eye Research Institute, Singapore	Performed statistical analysis; acquired data.
David J Stott	University of Glasgow, Glasgow, United Kingdom	Acquired data.
Henry Houlden	University College London, London, United Kingdom	Acquired data.
Reinhold Schmidt	Medical University of Graz, Graz, Austria	Acquired data.
Rebecca F Gottesman	Johns Hopkins University, Baltimore, United States of America	Acquired data.
Andrew D MacKinnon	Atkinson Morley Neurosciences Centre, London, United Kingdom	Acquired data.
Charles	Boston University, Boston, United	Acquired data.

DeCarli	States of America	
Vilmundur Gudnason	Icelandic Heart Association, Kopavogur, Iceland	Acquired data.
Ian J Deary	University of Edinburgh, Edinburgh, United Kingdom	Acquired data.
Cornelia M van Duijn	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data.
P Eline Slagboom	Leiden University Medical Center, Leiden, the Netherlands	Acquired data.
Tien Yin Wong	Singapore Eye Research Institute, Singapore	Acquired data.
Natalia S Rost	Massachusetts General Hospital, Boston, United States of America	Acquired data.
J Wouter Jukema	Leiden University Medical Center, Leiden, the Netherlands	Acquired data.
Thomas H Mosley	University of Mississippi, Jackson, United States of America	Acquired data.
David J Werring	University College London, London, United Kingdom	Acquired data.
Helena Schmidt	Medical University of Graz, Graz, Austria	Acquired data.
Joanna M Wardlaw	University of Edinburgh, Edinburgh, United Kingdom	Acquired data.
M Arfan Ikram	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data; directed the work.

Sudha Seshadri	UT Health San Antonio, San Antonio, United States of America	Acquired data; directed the work.
Lenore J Launer	National Institutes of Health, Baltimore, United States of America	Acquired data; directed the work.
Hugh S Markus	University of Cambridge, Cambridge, United Kingdom	Acquired data; drafted the manuscript; directed the work.

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