Association of common genetic variants with brain microbleeds: a genome-wide association study

Knol, M.J.; Lu, D.W.; Traylor, M.; Adams, H.H.H.; Romero, J.R.J.; Smith, A.V.; ... ; Markus, H.S.

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Association of common genetic variants with brain microbleeds

A genome-wide association study

Maria J. Knol, BSc,* Dongwei Lu, MD, PhD,* Matthew Traylor, PhD,* Hieab H.H. Adams, MD, PhD,* José Rafael J. Romero, MD, Albert V. Smith, PhD, Myriam Fornage, PhD, Edith Hofer, PhD, Junfeng Liu, MD, PhD, Isabel C. Hostettler, MD, Michelle Luciano, PhD, Stella Trompet, PhD, Anne-Katrin Giese, MD, Saima Hilal, MD, PhD, Erik B. van den Akker, PhD, Dina Vojinovic, MD, PhD, Shuo Li, PhD, Sigurdur Sigurdsson, MSc, Sven J. van der Lee, MD, PhD, Clifford R. Jack, Jr., MD, Duncan Wilson, PhD, Pinar Yilmaz, MD, Claudia L. Satizabal, PhD, David C.M. Liewald, BSc, Jeroen van der Grond, PhD, Christopher Chen, FRCP, Yasaman Saba, MSc, Aad van der Lugt, MD, PhD, Mark E. Bastin, DPhil, B. Gwen Windham, MD, MHS, Ching Yu Cheng, MD, PhD, Lukas Pirpamer, MSc, Kejal Kantarci, MD, Jayandra J. Himali, PhD, Qiong Yang, PhD, Zoe Morris, MD, Alexa S. Beiser, PhD, Daniel J. Tozer, PhD, Meike W. Vernooij, MD, PhD, Najaf Amin, PhD, Marian Beekman, PhD, Jia Yu Koh, PhD, David J. Stott, MD, Henry Houlden, PhD, Reinhold Schmidt, MD, Rebecca F. Gottesman, MD, PhD, Andrew D. MacKinnon, MD, Charles DeCarli, MD, Vilmundur Gudnason, MD, PhD, Ian J. Deary, PhD, Cornelia M. van Duijn, PhD, P. Eline Slagboom, PhD, Tien Yin Wong, MD, PhD, Natalia S. Rost, MD, MPH, J. Wouter Jukema, PhD, Thomas H. Mosley, PhD, David J. Werring, PhD, Helena Schmidt, MD, PhD, Joanna M. Wardlaw, MD, M. Arfan Ikram, MD, PhD,† Sudha Seshadri, MD,† Lenore J. Launer, PhD,† and Hugh S. Markus, DM, FMed Sci,† for the Alzheimer's Disease Neuroimaging Initiative

Correspondence
Dr. Launer
launerl@nia.nih.gov
or Dr. Markus
hsm32@medschl.cam.ac.uk

Neurology® 2020;95:e3331-e3343. doi:10.1212/WNL.0000000000010852

Abstract

Objective

To identify common genetic variants associated with the presence of brain microbleeds (BMBs).

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

*These authors contributed equally to this work.

†These authors jointly directed the work.

From the Departments of Epidemiology (M.J.K., H.H.H.A., D.V., S.J.v.d.L., P.Y., M.W.V., N.A., C.M.v.D., M.A.I.), Radiology and Nuclear Medicine (H.H.H.A., P.Y., A.v.d.L., M.W.V.), and Clinical Genetics (H.H.H.A.), Erasmus MC University Medical Center, Rotterdam, the Netherlands; Stroke Research Group, Department of Clinical Neurosciences (D.L., M.T., J.L., D.J.T., H.S.M.), University of Cambridge, UK; Department of Neurology (J.R.J.R., C.L.S., J.J.H., A.S.B., C.D., S. Seshadri), Boston University School of Medicine; The Framingham Heart Study (J.R.J.R., C.L.S., J.J.H., A.S.B., S. Seshadri), MA; Department of Biostatistics (A.V.S.), University of Michigan, Ann Arbor; Icelandic Heart Association (A.V.S., S. Sigurdsson, V.G.), Kopavogur, Iceland; Brown Foundation Institute of Molecular Medicine, McGovern Medical School (M.F.), and Human Genetics Center, School of Public Health (M.F.), University of Texas Health Science Center at Houston; Clinical Division of Neurogeriatrics, Department of Neurology (E.H., L.P., R.S.), Institute for Medical Informatics, Statistics and Documentation (E.H.), and Gottfried Schatz Research Center, Department of Molecular Biology and Biochemistry (Y.S., H.S.), Medical University of Graz, Austria; Center of Cerebrovascular Diseases, Department of Neurology (J.L.), West China Hospital, Sichuan University, Chengdu; Stroke Research Centre, Queen Square Institute of Neurology (I.C.H., D.W., H.H., D.J.W.), University College London, UK; Department of Neurosurgery (I.C.H.), Klinikum rechts der Isar, University of Munich, Germany; Centre for Cognitive Ageing and Cognitive Epidemiology, Psychology (M.L., D.C.M.L., M.E.B., I.J.D., J.M.W.), and Centre for Clinical Brain Sciences, Edinburgh Imaging, UK Dementia Research Institute (M.E.B., J.M.W.), University of Edinburgh, UK; Department of Internal Medicine, Section of Gerontology and Geriatrics (S.T.), Department of Cardiology (S.T., J.v.d.G., J.W.J.), Section of Molecular Epidemiology, Biomedical Data Sciences (E.B.v.d.A., M.B., P.E.S.), Leiden Computational Biology Center, Biomedical Data Sciences (E.B.v.d.A.), Department of Radiology (J.v.d.G.), and Einthoven Laboratory for Experimental Vascular Medicine (J.W.J.), Leiden University Medical Center, the Netherlands; Department of Neurology (A.-K.G., N.S.R.), Massachusetts General Hospital, Harvard Medical School, Boston; Memory Aging and Cognition Center (S.H., C.C.), National University Health System, Singapore; Department of Pharmacology (S.H., C.C.) and Saw Swee Hock School of Public Health (S.H.), National University of Singapore and National University Health System, Singapore; Pattern Recognition & Bioinformatics (E.B.v.d.A.), Delft University of Technology, the Netherlands; Department of Biostatistics (S.L., J.J.H., Q.Y., A.S.B.), Boston University School of Public Health, MA; Department of Radiology (C.R.J., K.K.), Mayo Clinic, Rochester, MN; Glenn Biggs Institute for Alzheimer's & Neurodegenerative Diseases (C.L.S., S. Seshadri), UT Health San Antonio, TX; Department of Medicine, Division of Geriatrics (B.G.W., T.H.M), and Memory Impairment and Neurodegenerative Dementia (MIND) Center (T.H.M.), University of Mississippi Medical Center, Jackson; Singapore Eye Research Institute (C.Y.C., J.Y.K., T.Y.W.); Department of Neuroradiology (Z.M., J.M.W.), NHS Lothian, Edinburgh; Institute of Cardiovascular and Medical Sciences (D.J.S.), College of Medical, Veterinary and Life Sciences, University of Glasgow, UK; Division of Cerebrovascular Neurology (R.F.G.), Johns Hopkins University, Baltimore, MD; Department of Neuroradiology (A.D.M.), Atkinson Morley Neurosciences Centre, St George's NHS Foundation Trust, London, UK; Department of Neurology (C.D.), University of California at Davis; Nuffield Department of Population Health (C.M.v.D.), University of Oxford, UK; Laboratory of Epidemiology and Population Sciences (L.J.L.), National Institute on Aging, Baltimore, MD; and Faculty of Medicine (V.G.), University of Iceland, Reykjavik, Iceland.

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found in the coinvestigators list at links.lww.com/WNL/B222.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Glossary

AD = Alzheimer disease; **CHARGE** = Cohorts of Heart and Aging Research in Genomic Epidemiology; **CI** = confidence interval; **CSVD** = cerebral small vessel disease; **BMB** = brain microbleed; **GWAS** = genome-wide association studies; **ICH** = intracerebral hemorrhage; **LD** = linkage disequilibrium; **MAF** = minor allele frequency; **OR** = odds ratio; **SNP** = single nucleotide polymorphism; **SWI** = susceptibility-weighted imaging; **WMH** = white matter hyperintensities.

Consortium or 1000 Genomes reference panel. BMBs were rated on susceptibility-weighted or T2*-weighted gradient echo MRI 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 BMBs.

Results

BMBs 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 *single nucleotide polymorphism* rs769449; odds ratio $[OR]_{any\ BMB}$ [95% confidence interval (CI)] 1.33 [1.21–1.45]; $p = 2.5 \times 10^{-10}$). *APOE* ε4 alleles were associated with strictly lobar (OR [95% CI] 1.34 [1.19–1.50]; $p = 1.0 \times 10^{-6}$) 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 hemorrhage 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 BMBs. Genetic predisposition to small vessel disease confers risk of BMB, indicating genetic overlap with other cerebral small vessel disease markers.

Brain microbleeds (BMBs), also referred to as cerebral microbleeds or cerebral microhemorrhages, correspond to hemosiderin deposits as a result of microscopic hemorrhages that are visible on MRI sequences.[1] The frequency of BMBs increases with age and with certain pathologies, including cerebral small vessel disease (CSVD),[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 the cortical area or the cortico-subcortical border (lobar) and the subcortical (deep) structures of the brain. BMBs in lobar regions are often seen in both familial and sporadic cerebral amyloid angiopathy, whereas deep BMBs are more common in sporadic deep perforator arteriopathy.[6–8] This suggests that different pathophysiologic mechanisms may underlie BMBs in the 2 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]

BMBs represent one of a spectrum of MRI markers of CSVD, 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 2 large initiatives: the Cohorts of Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium[14] and the UK Biobank (ukbiobank.ac.uk), combined with additional data from the case–control Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu) and the Massachusetts General Hospital Genes Affecting Stroke Risk and Outcomes Study (MGH-GASROS)[15] and Clinical Relevance of Microbleeds in Stroke due to Atrial Fibrillation (CROMIS-2 AF)[4] stroke studies. Together this comprised 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 ethics committees. Written informed consent was obtained from all individuals participating in the study.

Table 1 Population characteristics of contributing studies

Study	Study design	Ancestry	Total	Any BMBs	Lobar BMBs	Mixed BMBs	Female	Age, y	Age range, y	Dementia	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
Totals			25,862	3,556	2,179	1,293					

Abbreviations: AA = African ancestry; AD = Alzheimer disease; ADNI = Alzheimer's Disease Neuroimaging Initiative; AGES = Age, Gene/Environment Susceptibility–Reykjavik Study; ARIC = Atherosclerosis Risk in Communities; ASPS = Austrian Stroke Prevention Study; BMB = brain microbleed; 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; PROSPER = Prospective Study of Pravastatin in the Elderly at Risk; RCT = randomized controlled trial; RS = Rotterdam Study; SCES = Singapore Chinese Eye Study; SiMES = Singapore Malay Eye Study.
Values are n (%) or mean ± SD.

Genotyping

Genotyping was performed on commercially available assays from Illumina (San Diego, CA) or Affymetrix (Santa Clara, CA) and were imputed using the Haplotype Reference Consortium or 1000 Genomes reference panels (supplementary table e-1, doi.org/10.5061/dryad.mcvdncjz4). Most cohorts included individuals of European ancestry only, but a subset of individuals with Chinese, Malay, or 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 e-2, doi.org/10.5061/dryad.mcvdncjz4). Definitions of BMB have been described previously.[16] Briefly, BMBs 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] Because previous research has shown differences between risk factors and clinical correlates of BMBs in specific locations of the brain,[6,8,20] we further differentiated between strictly lobar and deep infratentorial or mixed BMBs. Cases in which there were microbleeds located in cortical gray or subcortical white matter of the brain lobes without any microbleeds in deep or infratentorial regions were classified as lobar BMBs. Microbleeds in the deep gray matter of basal ganglia and thalamus or in brainstem or cerebellum were classified as deep or infratentorial BMBs. 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 (table 1), the data on lobar or mixed BMB were not available, and therefore the total number of lobar and mixed BMBs is slightly less than the total number of BMBs. Study-specific methodologies for the identification of BMBs have been described elsewhere.[1,6,21–30] Because 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 (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 using an INFO or r^2 above 0.5, minor allele frequency (MAF) above 0.005, and MAF*N_{cases}*imputation quality > 5. Within the CHARGE consortium plus additional case–control and case-only studies, only variants available in at least 2 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 linkage disequilibrium (LD) score regression intercept (supplementary table e-3, 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 ($p < 5 \times 10^{-6}$) and genome-wide significant ($p < 5 \times 10^{-8}$) variants, and variants in LD at a threshold of $r^2 > 0.8$.[33]

APOE ε2 and ε4 count analysis

In the 2 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 disease (AD) 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 BMBs, we performed a 2-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 that in turn might be related to BMBs, we assessed the association with BMBs as well. First we examined variants associated with other manifestations of CSVD, namely WMH,[10,11,15] lacunar stroke,[38,39] and ICH.[39,40] Second we examined associations with traits that have been shown to be predicted by BMB, namely any stroke, any ischemic stroke,[41,42] and AD.[43] For each related phenotype, we corrected

Table 2 Independent genetic variants significantly ($p < 5 \times 10^{-8}$) or suggestively ($p < 1 \times 10^{-6}$) associated with any or location-specific brain microbleeds (BMBs)

SNP	Chr	Position	A1	A2	EAF	Nearest gene	Outcome	β	SE	OR	Total	Cases	*p* Value
rs769449	19	45410002	A	G	0.13	*APOE*	Any BMBs	0.282	0.045	1.33	20,150	2,858	2.5×10^{-10}
							Lobar BMBs	0.280	0.055	1.32	18,666	1,748	4.3×10^{-7}
							Mixed BMBs	0.243	0.070	1.27	18,319	1,049	5.4×10^{-4}
rs6950978	7	87200467	A	T	0.70	*ABCB1*	Any BMBs	−0.154	0.030	0.86	25,528	3,439	2.7×10^{-7}
							Lobar BMBs	−0.153	0.037	0.86	24,101	2,101	4.1×10^{-5}
							Mixed BMBs	−0.179	0.046	0.84	23,033	1,239	1.0×10^{-4}
rs7533718	1	22281393	A	G	0.83	*HSPG2*	Any BMBs	−0.140	0.042	0.87	25,402	3,412	7.5×10^{-4}
							Lobar BMBs	−0.263	0.051	0.77	22,935	2,005	2.9×10^{-7}
							Mixed BMBs	0.003	0.070	1.00	22,446	1,161	9.7×10^{-1}
rs11025317	11	3103445	A	G	0.12	*OSBPL5*	Any BMBs	0.172	0.049	1.19	20,330	2,918	4.3×10^{-4}
							Lobar BMBs	0.305	0.060	1.36	18,666	1,748	3.0×10^{-7}
							Mixed BMBs	−0.027	0.082	0.97	17,714	996	7.4×10^{-1}
rs62522567	8	103799094	A	G	0.92	*GASAL1*	Any BMBs	−0.231	0.051	0.79	24,118	3,115	6.9×10^{-6}
							Lobar BMBs	−0.319	0.063	0.73	22,550	1,924	4.0×10^{-7}
							Mixed BMBs	−0.195	0.089	0.82	17,075	942	2.8×10^{-2}
rs1058285	19	43680051	T	C	0.61	*PSG5*	Any BMBs	0.082	0.030	1.08	24,794	3,290	6.0×10^{-3}
							Lobar BMBs	0.188	0.038	1.21	23,535	2,021	5.3×10^{-7}
							Mixed BMBs	−0.051	0.045	0.95	22,729	1,216	2.6×10^{-1}
rs654240	11	69448373	T	C	0.41	*CCND1*	Any BMBs	0.154	0.031	1.17	25,402	3,412	7.4×10^{-7}
							Lobar BMBs	0.116	0.039	1.12	23,528	2,080	2.8×10^{-3}
							Mixed BMBs	0.202	0.048	1.22	23,368	1,270	3.0×10^{-5}

Abbreviations: A1 = effect allele; A2 = other allele; Chr = chromosome; EAF = effect allele frequency; OR = odds ratio; SNP = single nucleotide polymorphism. Associations with BMBs with a $p < 1 \times 10^{-6}$. If available, the associations of the same genetic variants in the other analyses are also shown.

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 BMBs 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

The summary statistics will be made available upon publication on the CHARGE dbGaP site under the accession number phs000930.v7.p1 and via the Cerebrovascular Disease Knowledge Portal (cerebrovascularportal.org).

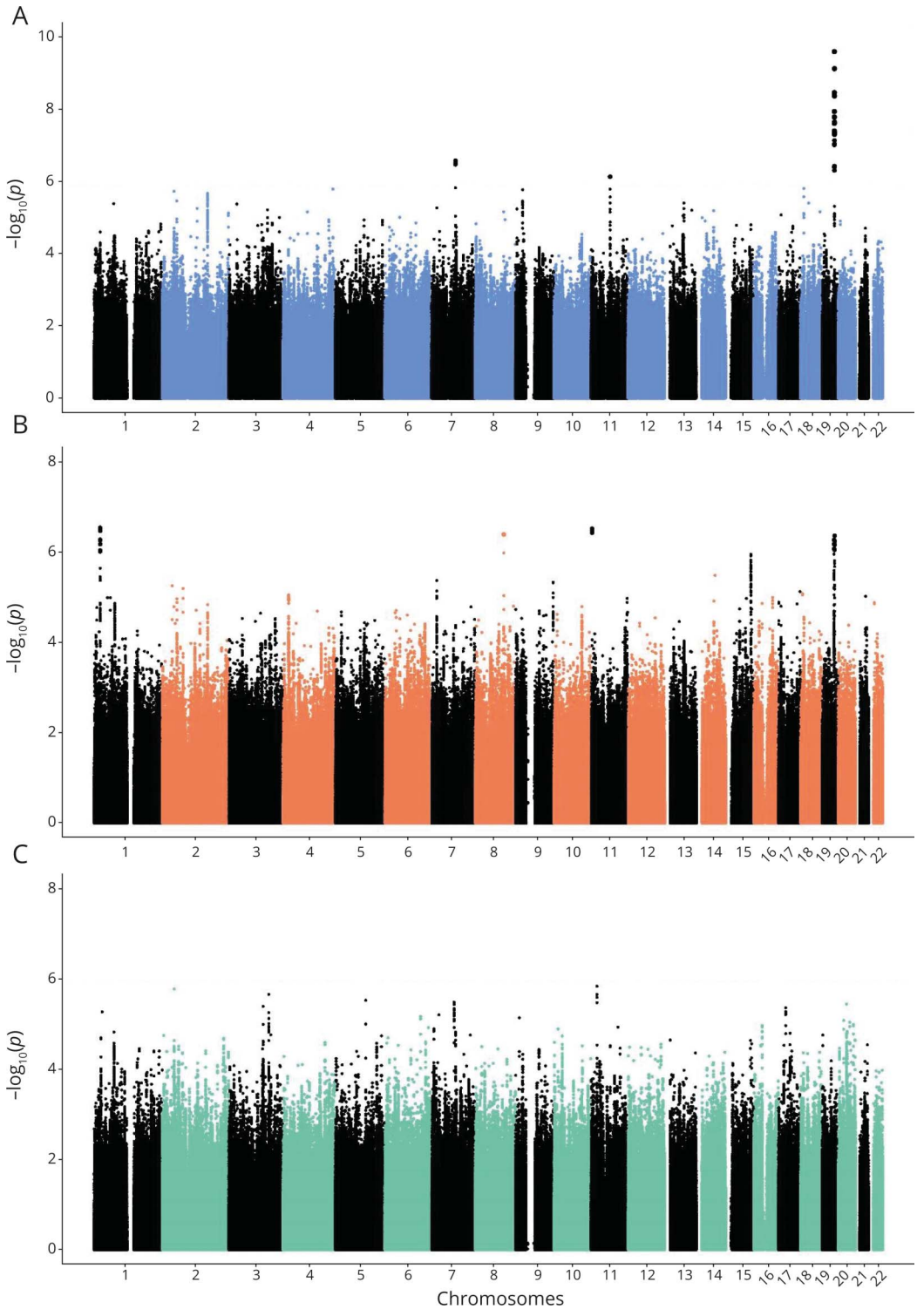
Results

In the combined CHARGE with additional studies and UK Biobank multiethnic meta-analysis, genetic and BMB rating data were available for 25,862 participants, 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 participants 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 e-1, doi.org/10.5061/dryad.mcvdncjz4), and limited genomic inflation was observed (λ = 1.02, LD score regression intercept = 1.02, supplementary table e-3, doi.org/10.5061/dryad.mcvdncjz4). One locus in the *APOE* region on chromosome 19 reached genome-wide significance (lead genetic variant

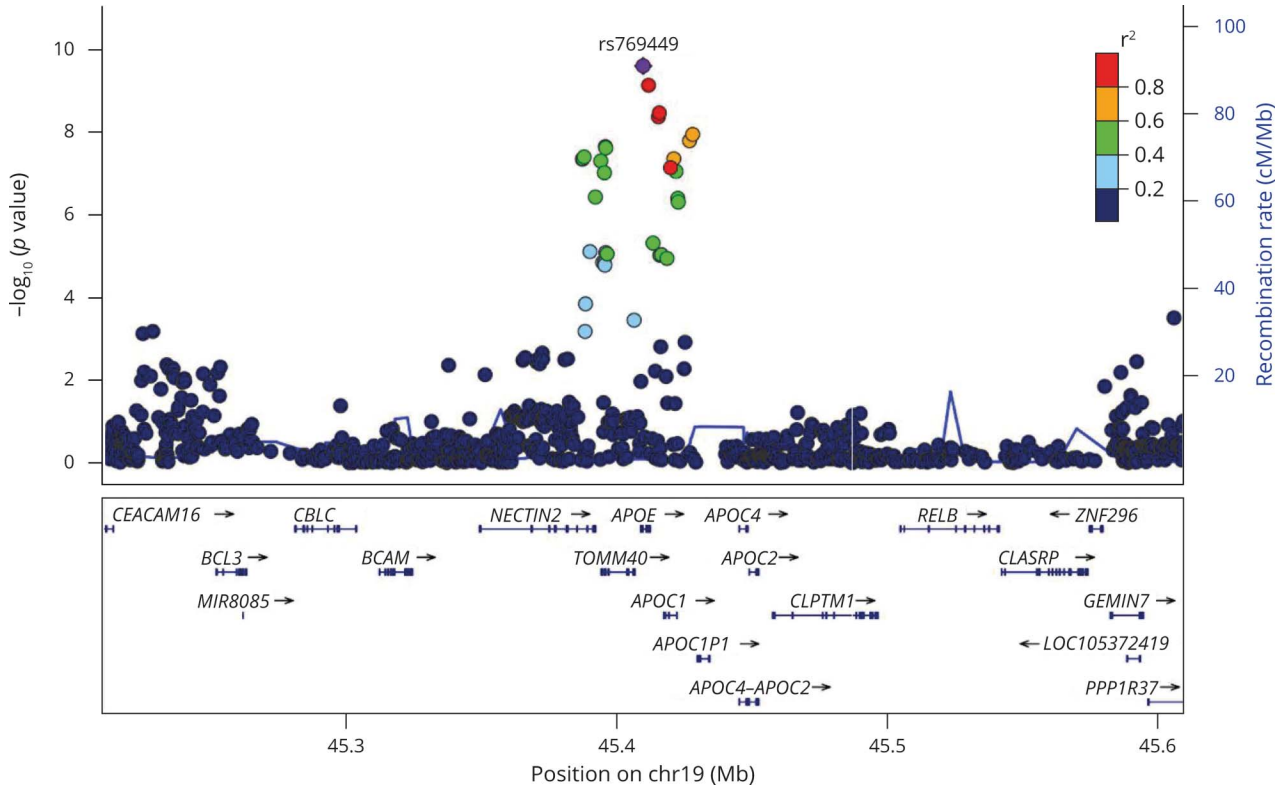
Figure 1 Common genetic variants associated with brain microbleeds



Manhattan plots show genome-wide associations by chromosomal position for (A) any, (B) lobar, and (C) mixed microbleeds.

rs769449; odds ratio [OR] [95% confidence interval (CI)] 1.33 [1.21–1.45]; $p = 2.5 \times 10^{-10}$; table 2, figures 1 and 2, and supplementary figure e-2, doi.org/10.5061/dryad.mcvdncjz4). This effect was stronger for lobar (OR [95% CI] 1.32 [1.19–1.47]; $p = 4.3 \times 10^{-7}$) than for mixed microbleeds (OR [95% CI] 1.27 [1.11–1.46]; $p = 5.4 \times 10^{-4}$), albeit not significantly. Similar associations were observed for the different participating studies (CHARGE with additional studies $I^2 = 0$, $p_{\text{heterozygosity}} = 0.68$; CHARGE with additional studies and UK Biobank combined $I^2 = 0$, $p_{\text{heterozygosity}} = 0.78$, supplementary figure e-3, doi.org/10.5061/dryad.mcvdncjz4). Functional annotation of the genome-wide significant variants and

Figure 2 Regional association of genome-wide significant locus for any brain microbleeds



Regional plot shows association of genetic variants in the *APOE* region with any brain microbleeds.

genetic variants in LD ($r^2 > 0.8$) are presented in supplementary table e-4, 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], $p = 2.1 \times 10^{-8}$, supplementary table e-5 and supplementary figure e-4, doi.org/10.5061/dryad.mcvdncjz4).

APOE ε2 and ε4 count analysis

To further elucidate whether 1 of the 2 *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 2 largest cohorts (Rotterdam Study and UK Biobank). The *APOE* ε4 allele count was significantly associated with the number of BMBs (OR [95% CI] 1.27 [1.14–1.42]; $p = 1.3 \times$

Table 3 The effects of *APOE* ε2 and ε4 allele count on the number of brain microbleeds (BMBs) overall and by location

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

Abbreviations: CI = confidence interval; OR = odds ratio.

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 to 0.031)	0.170
Systolic blood pressure	0.026 (0.005 to 0.046)	0.013[a]
Diastolic blood pressure	0.046 (0.010 to 0.082)	0.011[a]
Pulse pressure	0.021 (−0.008 to 0.049)	0.156
Body mass index	−0.037 (−0.131 to 0.057)	0.445
Low density lipoprotein	0.057 (−0.085 to 0.198)	0.431
High density lipoprotein	−0.001 (−0.159 to 0.157)	0.990
Triglycerides	0.290 (0.090 to 0.489)	0.004[b]
Lobar brain microbleeds		
Type 2 diabetes	−0.053 (−0.180 to 0.074)	0.414
Systolic blood pressure	0.027 (0.003 to 0.051)	0.029[a]
Diastolic blood pressure	0.046 (0.003 to 0.088)	0.035[a]
Pulse pressure	0.023 (−0.010 to 0.057)	0.174
Body mass index	−0.023 (−0.141 to 0.094)	0.697
Low density lipoprotein	0.145 (−0.015 to 0.306)	0.076
High density lipoprotein	−0.024 (−0.206 to 0.159)	0.799
Triglycerides	0.250 (0.015 to 0.486)	0.037[a]
Mixed brain microbleeds		
Type 2 diabetes	−0.074 (−0.222 to 0.073)	0.323
Systolic blood pressure	0.024 (−0.005 to 0.054)	0.108
Diastolic blood pressure	0.034 (−0.019 to 0.086)	0.209
Pulse pressure	0.025 (−0.017 to 0.066)	0.243
Body mass index	−0.047 (−0.191 to 0.097)	0.524
Low density lipoprotein	−0.078 (−0.315 to 0.159)	0.519
High density lipoprotein	−0.050 (−0.263 to 0.162)	0.642
Triglycerides	0.374 (0.094 to 0.654)	0.009[a]

Abbreviation: CI = confidence interval.
[a] Nominally significant associations ($p < 0.05$).
[b] Significant after adjustment for the number of risk factors ($p <$ [0.05/8]).

10^{-5}; table 3). This effect was stronger for lobar than for mixed microbleeds (OR [95% CI] 1.33 [1.16–1.52]; $p = 3.5 \times 10^{-5}$ 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 e-6, doi.org/10.5061/dryad.mcvdncjz4). No significant association was found between the *APOE* ε2 allele count and the number of BMBs (OR [95% CI] 1.03 [0.86–1.22]; $p = 0.769$), also not after removing individuals with the *APOE* ε2ε4 genotype (table 3 and supplementary table e-6, doi.org/10.5061/dryad.mcvdncjz4).

Two-sample mendelian randomization

Mendelian randomization analyses testing the influence of cardiovascular risk factors on BMBs 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 BMBs as well as triglycerides with deep, infratentorial, or mixed BMBs (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], $p = 1.8 \times 10^{-4}$), with slightly stronger effects on mixed BMB than lobar BMB (OR [95% CI] 1.14 [1.05–1.25], $p = 3.2 \times 10^{-3}$ vs OR [95% CI] 1.09 [1.01–1.17], $p = 2.2 \times 10^{-2}$) (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 CSVD variants were individually associated with BMB. 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 AD and stroke are presented in supplementary table e-7 (doi.org/10.5061/dryad.mcvdncjz4).

Discussion

We report the first large-scale multiethnic genome-wide study of BMBs in 25,862 individuals, including 3,556 participants 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 BMBs, 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 1 of the 2 SNPs constituting *APOE* ε2/3/4 polymorphisms; this variant was more strongly associated with strictly lobar than mixed BMB. In an

Table 5 Association of cerebral small vessel disease–associated genetic variants with brain microbleeds (BMBs) overall and by location

			All BMBs		Lobar BMBs		Mixed BMBs	
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[a]	1.09 (1.01–1.17)	0.022[a]	1.14 (1.05–1.25)	0.003[a]
	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[b]	1.04 (0.97–1.12)	0.277	1.10 (1.00–1.20)	0.039[b]
	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[a]
WMH[c]	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[a]	1.09 (1.01–1.17)	0.022[b]	1.14 (1.05–1.25)	0.003[a]
	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[b]	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[b]
	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[b]

Abbreviations: CI = confidence interval; ICH = intracerebral hemorrhage; OR = odds ratio; SNP = single nucleotide polymorphism; WMH = white matter hyperintensities.
ORs aligned to risk allele from original studies.
[a] Significant after Bonferroni correction ($p < 0.05$/number of genetic variants).
[b] Nominally significant ($p < 0.05$).
[c] In the overall score for WMH, rs12357919 was left out because this genetic variant was in linkage disequilibrium ($r^2 > 0.2$) with rs72848980.

additional analysis performed in a subset of the cohorts, we confirmed the known link between *APOE* ε4 allele count and the number of BMBs, with stronger effect estimates for the strictly lobar BMB subtype compared to the mixed subtype. This association was less pronounced and nonsignificant for the *APOE* ε2 allele count, which is also in accordance with previous studies,[12] although this might 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 BMBs in the current study might be due to sampling variability or biological differences between the 2 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 individuals with disease, 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 BMBs. This relationship between the genetics of triglycerides and BMBs, in particular for mixed BMBs, confirms other studies showing a contribution of cardiovascular risk factors to BMB risk, mainly for deep or infratentorial BMBs.[6] A previous 2-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 BMBs in the current study.[44] However, this positive link between the genetics of triglyceride levels and the presence of BMBs 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 CSVD (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 BMBs have a shared pathophysiologic pathway with other features of the CSVD spectrum. This is consistent with recent data showing genetic sharing between WMH, lacunar infarcts, and subcortical ICH.[49] Increasing evidence suggests that small vessel arteriopathy may lead to WMH, acute lacunar infarction, and ICH.[50] Our data suggest that mixed BMBs 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 AD pathology conferred by *APOE* ε4, our results suggest that another part might be due to vascular mechanisms predisposing to BMBs, most likely via cerebral amyloid angiopathy. Apart from the *APOE* locus, no enrichment of previously reported genetic variants for AD 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 AD.[11] It might indicate that *APOE* is mainly responsible for the genetic overlap between BMB and AD. Alternatively, the current BMB and AD GWAS could be underpowered to identify biological pathways playing a role in the development of CSVD subsequently leading to AD. 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 BMBs, enabling us to perform by far the largest GWAS meta-analysis of BMB to date. 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 BMBs, we combined the presence of deep, infratentorial, and mixed BMBs into one group of mixed BMBs, even though previous research has suggested there may be differences between strictly deep and mixed BMBs.[20] With larger sample sizes, it would be interesting to investigate whether there are differences in the genetics between deep and infratentorial BMBs. The percentage of individuals with microbleeds varied across studies, which may be due to a true difference in the presence of BMBs or population differences, e.g., age distributions, ethnicities, and lifestyle factors. However, the differences in the presence of BMBs 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. Another limitation of the current study is the large majority of individuals of European ancestry included in the analyses; previous studies have shown differences in the occurrence, distribution, and associated risks of BMBs across different ethnicities.[52–54] Therefore, it would be valuable for future studies to increase the sample size of individuals of non-European ancestry 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.

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 CSVD, emphasizing that they represent part of the CSVD spectrum.

Study funding

This study was funded by the European Union's Horizon 2020 Framework Programme for Research and Innovation (grant 347 agreement 667375, CoSTREAM). Information regarding funding and acknowledgements for individual cohorts is provided in the Supplementary information (doi.org/10.5061/dryad.mcvdncjz4).

Disclosure

This study was not industry sponsored. M.J. Knol, D. Lu, and M. Traylor report no disclosures relevant to the manuscript. H.H.H. Adams is supported by ZonMW grant 916.19.151. J.R.J. Romero, A.V. Smith, M. Fornage, E. Hofer, and J. Liu report no disclosures relevant to the manuscript. I.C. Hostettler received funding from the Alzheimer Research UK and Dunhill Medical Trust Foundation. M. Luciano, S. Trompet, A.-K. Giese, S. Hilal, E.B. van den Akker, D. Vojinovic, S. Li, S. Sigurdsson, S.J. van der Lee, and C.R. Jack, Jr. report no disclosures relevant to the manuscript. D. Wilson received funding from the Stroke Foundation/British Heart Foundation. P. Yilmaz, C.L. Satizabal, D.C.M. Liewald, J. van der Grond, C. Chen, Y. Saba, A. van der Lugt, M.E. Bastin, B.G. Windham, C.Y. Cheng, L. Pirpamer, K. Kantarci, J.J. Himali, Q. Yang, Z. Morris, A.S. Beiser, D.J. Tozer, M.W. Vernooij, N. Amin, M. Beekman, J.Y. Koh, and D.J. Stott report no disclosures relevant to the manuscript. H. Houlden received funding from the Alzheimer Research UK and Dunhill Medical Trust Foundation. R. Schmidt, R.F. Gottesman, and A.D. MacKinnon report no disclosures relevant to the manuscript. C. DeCarli is supported by the Alzheimer's Disease Center (P30 AG 010129) and serves as a consultant of Novartis Pharmaceuticals. V. Gudnason, I.J. Deary, C.M. van Duijn, P.E. Slagboom, T.Y. Wong, and N.S. Rost report no disclosures relevant to the manuscript. J.W. Jukema is an Established Clinical Investigator of the Netherlands Heart Foundation (grant 2001 D 032). T.H. Mosley reports no disclosures relevant to the manuscript. D.J. Werring received

funding from the Stroke Foundation/British Heart Foundation. H. Schmidt, J.M. Wardlaw, M.A. Ikram, S. Seshadri, L.J. Launer, and H.S. Markus report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Publication history

Received by *Neurology* December 16, 2019. Accepted in final form August 3, 2020.

Appendix Authors

Name	Location	Contribution
Maria J. Knol, BSc	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Performed statistical analysis, drafted the manuscript
Dongwei Lu, MD, PhD	University of Cambridge, UK	Acquired data
Matthew Traylor, PhD	University of Cambridge, UK	Performed statistical analysis, drafted the manuscript
Hieab H.H. Adams, MD, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Performed statistical analysis, acquired data, drafted the manuscript
José Rafael J. Romero, MD	Boston University, MA	Acquired data
Albert V. Smith, PhD	University of Michigan	Performed statistical analysis
Myriam Fornage, PhD	University of Texas, Houston	Performed statistical analysis
Edith Hofer, PhD	Medical University of Graz, Austria	Performed statistical analysis
Junfeng Liu, MD, PhD	University of Cambridge, UK	Acquired data
Isabel C. Hostettler, MD	University College London, UK	Performed statistical analysis, acquired data
Michelle Luciano, PhD	University of Edinburgh, UK	Performed statistical analysis
Stella Trompet, PhD	Leiden University Medical Center, the Netherlands	Performed statistical analysis
Anne-Katrin Giese, MD	Massachusetts General Hospital, Boston	Performed statistical analysis
Saima Hilal, MD, PhD	Memory Aging and Cognition Center, Singapore	Performed statistical analysis, acquired data
Erik B. van den Akker, PhD	Leiden University Medical Center, the Netherlands	Performed statistical analysis
Dina Vojinovic, MD, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Performed statistical analysis
Shuo Li, PhD	Boston University, MA	Performed statistical analysis
Sigurdur Sigurdsson, MSc	Icelandic Heart Association, Kopavogur, Iceland	Acquired data
Sven J. van der Lee, MD, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Performed statistical analysis
Clifford R. Jack, Jr., MD	Mayo Clinic, Rochester, MN	Acquired data
Duncan Wilson, PhD	University College London, UK	Acquired data
Pinar Yilmaz, MD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data
Claudia L. Satizabal, PhD	UT Health San Antonio	Performed statistical analysis
David C.M. Liewald, BSc	University of Edinburgh, UK	Acquired data
Jeroen van der Grond, PhD	Leiden University Medical Center, the Netherlands	Acquired data
Christopher Chen, FRCP	Memory Aging and Cognition Center, Singapore	Acquired data
Yasaman Saba, MSc	Medical University of Graz, Austria	Performed statistical analysis
Aad van der Lugt, MD, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data
Mark E. Bastin, PhD	University of Edinburgh, UK	Acquired data
B. Gwen Windham, MD	University of Mississippi Medical Center, Jackson	Acquired data
Ching-Yu Cheng, MD, PhD	Singapore Eye Research Institute	Acquired data
Lukas Pirpamer, MSc	Medical University of Graz, Austria	Acquired data
Kejal Kantarci, MD	Mayo Clinic, Rochester, MN	Acquired data
Jayandra J. Himali, PhD	Boston University, MA	Performed statistical analysis
Qiong Yang, PhD	Boston University, MA	Acquired data
Zoe Morris, MD	University of Edinburgh, UK	Acquired data
Alexa S. Beiser, PhD	Boston University, MA	Acquired data
Daniel J. Tozer, PhD	University of Cambridge, UK	Acquired data

Continued

Appendix *(continued)*

Name	Location	Contribution
Meike W. Vernooij, MD, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data
Najaf Amin, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data
Marian Beekman, PhD	Leiden University Medical Center, the Netherlands	Acquired data
Jia Yu Koh, PhD	Singapore Eye Research Institute	Performed statistical analysis, acquired data
David J. Stott, MD, PhD	University of Glasgow, UK	Acquired data
Henry Houlden, PhD	University College London, UK	Acquired data
Reinhold Schmidt, MD	Medical University of Graz, Austria	Acquired data
Rebecca F. Gottesman, MD, PhD	Johns Hopkins University, Baltimore, MD	Acquired data
Andrew D. MacKinnon, MD	Atkinson Morley Neurosciences Centre, London, UK	Acquired data
Charles DeCarli, MD	Boston University, MA	Acquired data
Vilmundur Gudnason, MD, PhD	Icelandic Heart Association, Kopavogur, Iceland	Acquired data
Ian J. Deary, PhD	University of Edinburgh, UK	Acquired data
Cornelia M. van Duijn, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data
P. Eline Slagboom, PhD	Leiden University Medical Center, the Netherlands	Acquired data
Tien Yin Wong, MD, PhD	Singapore Eye Research Institute	Acquired data
Natalia S. Rost, MD, MPH	Massachusetts General Hospital, Boston	Acquired data
J. Wouter Jukema, PhD	Leiden University Medical Center, Leiden, the Netherlands	Acquired data
Thomas H. Mosley, PhD	University of Mississippi Medical Center, Jackson	Acquired data
David J. Werring, PhD	University College London, UK	Acquired data
Helena Schmidt, MD, PhD	Medical University of Graz, Austria	Acquired data
Joanna M. Wardlaw, MD	University of Edinburgh, UK	Acquired data
M. Arfan Ikram, MD, PhD	Erasmus MC University Medical Center, Rotterdam, the Netherlands	Acquired data, directed the work
Sudha Seshadri, MD	UT Health San Antonio, San Antonio	Acquired data, directed the work
Lenore J. Launer, PhD	National Institutes of Health, Baltimore, MD	Acquired data, directed the work
Hugh S. Markus, DM, FMed Sci	University of Cambridge, UK	Acquired data, drafted the manuscript, directed the work

Appendix 2 Coinvestigators

Coinvestigators are listed at links.lww.com/WNL/B222

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