九州大学学術情報リポジトリ
Kyushu University Institutional Repository

Prevalence of and risk factors for cerebral microbleeds in a general Japanese elderly community

由比, 智裕

https://hdl.handle.net/2324/4110439

出版情報：Kyushu University, 2020, 博士（医学）, 課程博士
バージョン：
権利関係：Public access to the fulltext file is restricted for unavoidable reason (2)

Prevalence of and risk factors for cerebral microbleeds in a general Japanese elderly community

Authors: Tomohiro Yubi, MD; Jun Hata, MD, PhD; Tomoyuki Ohara, MD, PhD; Naoko Mukai, MD, PhD; Yoichiro Hirakawa, MD, PhD; Daigo Yoshida, PhD; Seiji Gotoh, MD, PhD; Naoki Hirabayashi, MD, PhD; Yoshihiko Furuta, MD; Tetsuro Ago, MD, PhD; Takanari Kitazono, MD, PhD; Yutaka Kiyohara, MD, PhD; Toshiharu Ninomiya, MD, PhD

Tomohiro Yubi, Jun Hata, Daigo Yoshida, Yoshihiko Furuta, Toshiharu Ninomiya: Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Tomoyuki Ohara: Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Naoko Mukai: Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Yoichiro Hirakawa, Seiji Gotoh, Tetsuro Ago, Takanari Kitazono: Department of Medicine and Clinical Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Naoki Hirabayashi: Department of Psychosomatic Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Yutaka Kiyohara: Hisayama Research Institute for Lifestyle Diseases, Fukuoka, Japan.

Title character count: 95

Number of references: 30

Number of tables: 5

Number of figure: 0

Word count abstract: 227

Word count paper: 2943

Supplemental Data: STROND statement, e-Appendix, Figure e-1, Tables e-1 to e-6

Corresponding Author: Jun Hata, MD, PhD

Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University

3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Phone: (+81) 92-642-6151; Fax: (+81) 92-642-4854

E-mail: junhata@eph.med.kyushu-u.ac.jp

Tomohiro Yubi yubi@med.kyushu-u.ac.jp

Jun Hata junhata@eph.med.kyushu-u.ac.jp

Tomoyuki Ohara ohara77@npsych.med.kyushu-u.ac.jp

Naoko Mukai n-mukai@eph.med.kyushu-u.ac.jp

Yoichiro Hirakawa you1@eph.med.kyushu-u.ac.jp

Daigo Yoshida dyoshida@eph.med.kyushu-u.ac.jp

Seiji Gotoh gotoh@kyumed.jp

Naoki Hirabayashi naokih@cephal.med.kyushu-u.ac.jp

Yoshihiko Furuta furuta@eph.med.kyushu-u.ac.jp

Tetsuro Ago agou@intmed2.med.kyushu-u.ac.jp

Takanari Kitazono kitazono@intmed2.med.kyushu-u.ac.jp

Yutaka Kiyohara kiyohara@hisayamalife.or.jp

Toshiharu Ninomiya nino@eph.med.kyushu-u.ac.jp

Statistical Analysis: conducted by Dr. Tomohiro Yubi, MD, Kyushu University

Search Terms: Intracerebral hemorrhage, Cohort studies, Prevalence studies, Risk factors in epidemiology, MRI

Author Contributions:

Tomohiro Yubi, study concept and design, analysis and interpretation of data, acquisition of data, writing of the manuscript.

Jun Hata, study concept and design, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content.

Tomoyuki Ohara, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content.

Naoko Mukai, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content.

Yoichiro Hirakawa, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content.

Daigo Yoshida, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content.

Seiji Gotoh, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content.

Naoki Hirabayashi, interpretation of data, acquisition of data, critical revision of the

manuscript for intellectual content.

Yoshihiko Furuta, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content.

Tetsuro Ago, interpretation of data, critical revision of the manuscript for intellectual content.

Takanari Kitazono, interpretation of data, critical revision of the manuscript for intellectual content, study supervision.

Yutaka Kiyohara, study concept and design, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content, study supervision.

Toshiharu Ninomiya, study concept and design, interpretation of data, acquisition of data, critical revision of the manuscript for intellectual content, study supervision.

Co-investigators: None

Acknowledgements: We thank the staff of the Division of Health and Welfare of Hisayama for their cooperation in this study. We also would like to gratefully and sincerely thank Professor Yoshinao Oda, Professor Toru Iwaki, and the staff of the Department of Anatomic Pathology and Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University, who provided insight and expertise in regard to the autopsy findings that greatly assisted the research.

Author Disclosures: All authors report no disclosures

Study Funded by Grants-in-Aid for Scientific Research (A) (JP16H02644, and JP16H02692), (B) (JP16H05850, JP16H05557, and JP17H04126), and (C) (JP15K09267, JP15K08738, JP15K09835, JP16K09244, JP17K09114, JP17K09113, and JP17K01853) from the Ministry

of Education, Culture, Sports, Science and Technology of Japan; by Health and Labour Sciences Research Grants of the Ministry of Health, Labour and Welfare of Japan (H25-Junkankitou [Seishuu]-Sitei-022, H29-Junkankitou [Seishuu]-Ippan-003, and H27-Shokuhin-[Sitei]-017); and by the Japan Agency for Medical Research and Development (dk0207025, ek0210082, gm0610007, ek0210083, km0405202, and ek0210080).

Abstract

Objective: To investigate the prevalence of and risk factors for cerebral microbleeds (CMBs) in a cross-sectional study of a general population of Japanese elderly.

Methods: In 2012, brain MRI scanning at 1.5 Tesla and comprehensive health examination were conducted for 1281 residents aged 65 years or older. CMBs were defined as ovoid hypointensity lesions less than 10 mm in diameter on T2*-weighted images and classified into deep/infratentorial and/or lobar CMBs. Age- and sex-specific and overall prevalence of CMBs were estimated, and the associations of traditional cardiovascular risk factors and apolipoprotein E (APOE) polymorphism with the presence of CMBs were examined using a logistic regression analysis.

Results: The crude prevalences of total, deep/infratentorial, and lobar CMBs were 18.7% (n=240), 13.5% (n=173), and 9.6% (n=123), respectively. The prevalence of total CMBs was 23.0% in men and 15.5% in women and increased with aging in both sexes (both *P* for trend <0.01). Hypertension was significantly associated with the presence of both deep/infratentorial and lobar CMBs. Lower serum total cholesterol was a significant risk factor for deep/infratentorial CMBs, but not for lobar CMBs, while APOE-ε4 carriers had a significantly higher likelihood only of lobar CMBs compared with non-carriers.

Conclusions: Our study suggests that approximately one of five Japanese elderly people have CMBs, and that risk factors for deep/infratentorial and lobar CMBs are different, indicating the distinct pathological backgrounds of these lesions.

Introduction

Cerebral microbleeds (CMBs) are round lesions with hypointensity on T2*-weighted images of the brain MRI.[1] CMBs are usually asymptomatic and thus are often identified incidentally in the brain MRI examinations for patients with stroke and dementia.[2] Histologically, CMBs represent tiny focal collections of hemosiderin adjacent to abnormal small vessels affected by mainly hypertensive or amyloid angiopathy.[2] CMBs with hypertensive angiopathy are thought to be mostly localized in deep or infratentorial brain areas, while CMBs with amyloid angiopathy are mainly located in cerebral lobar regions.[2] Therefore, risk factors for CMBs are likely to differ by their locations. Meanwhile, cerebrovascular disease in Japanese and other Asian populations is known to have a different genetic and environmental background compared with cerebrovascular disease in Western populations.[3,4] This suggests the possibility of distinct characteristics of CMBs between populations. Recently, the presence of CMBs has been considered to be an early imaging marker of bleeding-prone angiopathy, and thereby as a predictor of the development of symptomatic stroke, especially intracerebral hemorrhage, and dementia in the elderly.[2] Therefore, the epidemiological evidence for CMBs in elderly populations is considered to be useful for understanding and prevention of subsequent stroke and dementia. A number of observational studies performed in various locations around the world have reported the prevalence of CMBs[5-17] and their risk factors[5-16] in adult populations, but there have been limited studies addressing these issues in Western elderly populations,[9-12] and only a few prevalence studies of Asian elderly populations, including Japanese. The aims of the present study were to estimate the prevalence of CMBs and to explore their risk factors with consideration of the brain localization of CMBs in a general Japanese elderly population.

Methods

Study Participants

The Hisayama Study is a prospective cohort study of cerebro-cardiovascular diseases established in 1961 in the Town of Hisayama, a suburban community of the Fukuoka metropolitan area on Kyushu Island, Japan. The population of the town in 2010 was approximately 8400. The age and occupational distributions and nutrient intake levels in the residents of Hisayama have been very similar to those in the country of Japan as a whole.[18] The design of the MRI scan study in the town was previously described in detail.[19] Briefly, in 2012, a total of 1906 individuals (93.6%) among 2036 residents aged ≥65 years participated in a screening survey for cognitive impairment and activities of daily living (Figure e-1). This survey was mainly conducted at the municipal center for health promotion (Hisayama Health C&C Center) with a rental mobile MRI scanner, but surveys were also performed at home, hospitals, or nursing homes for 539 individuals who could not visit the center due to physical disability, dementia, severe diseases, or other causes. Among the 1367 participants who visited the center, an MRI examination was performed for 1342 individuals. In addition, we performed a comprehensive health examination for cardiovascular risk factors in the same year. After excluding 1 individual who refused to participate in the study, 41 who did not participate in the comprehensive health examination, 12 without T2*-weighted imaging, and 7 without blood sampling, the remaining 1281 individuals were enrolled in the present study.

Standard protocol approvals, registrations, and patient consents

This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Research. Written informed consent was obtained from all participants.

Brain MRI Examination

In the MRI examination, T2*-weighted imaging, T1-weighted imaging, T2-weighted imaging,

fluid attenuated inversion recovery (FLAIR) imaging, and magnetic resonance angiography of the brain were performed using a 1.5-Tesla MRI scanner (Intera Pulsar; Philips Medical Systems, Best, the Netherlands) with a multi-channel head coil.[19] T2*-weighted two-dimensional gradient echo images were acquired with the following parameters: repetition time 670 ms, echo time 23 ms, flip angle 18°, field of view 240×191 mm, acquisition matrix 256×163, slice thickness 5 mm, and slice gap 1.5 mm. CMBs were defined as ovoid hypointense lesions on T2*-weighted images measuring <10 mm in diameter and surrounded by brain parenchyma over at least half the circumference of the lesion. Hemorrhagic infarction and physiological calcification in the globus pallidus, which also showed hypointensity on the T2*-weighted images, were excluded. Sulcul flow voids from vessels were also ignored. Subjects with CMBs were categorized into a deep/infratentorial CMBs group (located in the basal ganglia, thalamus, brain stem, or cerebellum) and a lobar CMBs group (located in the cortical gray matter, or subcortical or periventricular white matter). Subjects with both deep/infratentorial and lobar CMBs were included in both groups. For the sensitivity analysis, the strictly lobar CMBs group included the subjects with lobar CMBs without deep/infratentorial CMBs. The presence and the location of CMBs were determined by two stroke neurologists who were blinded to the clinical information (agreement ratio: 83.9%; interrater reliability: κ=0.54). If there was a conflicting interpretation, a third investigator read the scan and made a final decision.

Lacunar infarction on MRI was defined as a small ischemic lesion (≥3 and <15 mm in diameter) in the basal ganglia, thalamus, or brain stem visible on both the T1-weighted image (as a hypointense lesion) and the T2-weighted image (as a hyperintense lesion) with a surrounding hyperintense rim on the FLAIR image, irrespective of the presence or absence of clinical symptoms. The amounts of white matter hyperintensity (WMH) in periventricular

and deep white matter regions of the brain were quantified into four grades, from grade 0 (absent) to grade 3 (severe), according to the Fazecas Scale,[20] using the FLAIR or T2-weighted images. Positive WMH was defined as grade 2 or 3 WMH in either periventricular or deep white matter or both.

Other Variables

Blood pressure was measured 3 times in a sitting position, and the mean values were used in the analysis. Hypertension was defined as a mean blood pressure ≥140/90 mmHg or use of antihypertensive agents. Plasma glucose levels were determined by the hexokinase method. Diabetes mellitus was defined as fasting plasma glucose ≥7.0 mmol/L, 2-h postload or postprandial plasma glucose ≥11.1 mmol/L, or use of antidiabetic medications. Serum total cholesterol levels were determined enzymatically. Hypercholesterolemia was defined as a serum total cholesterol ≥5.69 mmol/L or use of lipid-lowering agents. Body height and weight were measured in light clothing without shoes, and body mass index (BMI) was defined as the body weight (in kilograms) divided by the height (in meters) squared. Information regarding smoking habits and alcohol intake was self-reported and was categorized as current habitual use or not. Regular exercise was defined as engaging in sports at least three times a week during leisure time. Antithrombotic medication included both antiplatelets (aspirin, clopidogrel, ticlopidine, and cilostazol) and anticoagulants (warfarin and direct oral anticoagulants). The polymorphism of apolipoprotein E (APOE) was determined by genotyping two single nucleotide polymorphisms (rs429358 and rs7412) using a multiplex polymerase chain reaction–based Invader assay (Third Wave Technologies, Madison, WI) for 1,106 participants with genomic DNA samples.[21,22] Those who carried at least 1 copy of the ε4 allele were categorized as APOE-ε4 carriers. The subjects with at least 1 copy of the ε2 allele were defined as APOE-ε2 carriers. History of symptomatic stroke was

defined as any preexisting stroke events. All symptomatic stroke events were adjudicated based on physical examinations and all available clinical information, and classified into ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage as described previously.[18] Dementia was ascertained using the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition[23] as described previously.[19,24]

Statistical Analysis

The methods for statistical analysis are available at e-Appendix.

Results

Table 1 shows the baseline characteristics of the study subjects. The mean age was 75 years (standard deviation [SD] 7), and the proportion of men was 43.4%. The prevalences of hypertension and diabetes were 70.7% and 23.3%, respectively, and the mean value of serum total cholesterol levels was 5.11 mmol/L (SD 0.93). Among total participants, 19.4% took an antithrombotic medication, and 18.9% were APOE-ε4 carriers. Men were more likely to have diabetes, a smoking habit, a drinking habit, and antithrombotic use than women, whereas women had higher serum total cholesterol than men.

Table 2 presents the prevalence of any CMBs, deep/infratentorial CMBs, and lobar CMBs. Among the 1281 participants, 240 (18.7%) had at least one CMB on MRI and 109 (8.5%) had multiple CMBs. The prevalences of deep/infratentorial CMBs and lobar CMBs were 13.5% (n=173) and 9.6% (n=123), respectively. There were 56 subjects (4.4%) who had both deep/infratentorial and lobar CMBs, and 67 subjects (5.2%) who had strictly lobar CMBs without deep/infratentorial CMBs. The prevalence of any CMBs was 23.0% in men and 15.5% in women. The prevalence of any CMBs increased linearly with aging, from 11.8%

(age 65 to 69 years) to 29.2% (age 85 years or older), and the same was true for both deep/infratentorial and lobar CMBs (*P* for trend <0.01 for all, *P* for nonlinearity >0.5 for all). No evidence of an interaction between age and sex was found in the prevalence of any, deep/infratentorial, or lobar CMBs (*P* for interaction >0.3 for all).

As shown in Table 3, older age, male sex, hypertension, use of antithrombotic agents, and APOE-ε4 genotype were significantly associated with the presence of any CMBs in the age- and sex-adjusted analysis. In the multivariable-adjusted analysis, older age and hypertension remained independently significant risk factors for any CMBs, while the associations of sex, antithrombotic treatment, and APOE-ε4 genotype with the presence of any CMBs were no longer significant.

Tables 4 and 5 demonstrate the associations of each risk factor with deep/infratentorial CMBs and with lobar CMBs, respectively. In the multivariable-adjusted analysis, older age and hypertension were significantly associated with higher risk of the presence of both deep/infratentorial and lobar CMBs. Lower serum total cholesterol was a significant risk factor for deep/infratentorial CMBs, but not for lobar CMBs. In contrast, APOE-ε4 carriers had a significantly higher risk of the presence of lobar CMBs than non-carriers, but no clear association was observed between APOE genotype and deep/infratentorial CMBs. Men were more likely to have lobar CMBs than women even after adjustment for potential confounding factors. Similar associations were observed for strictly lobar CMBs without deep/infratentorial CMBs (Table e-1).

Table e-2 shows the prevalence of CMBs in APOE-ε4 carriers and non-carriers. APOE-ε4 carriers were more likely to have lobar CMBs than non-carriers.

Since hypertension was a risk factor for CMBs in any location, the associations of blood pressure levels and antihypertensive treatment status with the prevalence of CMBs are presented in Table e-3. Users of antihypertensive agents were more likely to have deep/infratentorial and lobar CMBs, and elevated BP levels were associated with the presence of deep/infratentorial CMBs.

Finally, Table e-4 shows the association of CMBs with other neurological disorders such as symptomatic stroke (ischemic stroke and intracerebral hemorrhage), dementia, and ischemic small vessel diseases (lacunar infarction and WHM) on MRI. The presence of any, deep/infratentorial, and lobar CMBs were all significantly associated with these neurological disorders.

Discussion

The present cross-sectional survey demonstrated that approximately one of five elderly people aged ≥65 years had CMBs on a brain MRI in a general Japanese population. The prevalence of deep/infratentorial CMBs was higher than that of lobar CMBs. Older age and hypertension were associated with both deep/infratentorial and lobar CMBs. While lower serum cholesterol levels were a risk factor for deep/infratentorial CMBs, the APOE-ε4 genotype and male sex were linked to the presence of lobar CMBs. The presence of CMBs was associated with other neurological disorders such as symptomatic stroke, dementia, and ischemic small vessel diseases on MRI.

The present study revealed that the prevalence of CMBs was 18.7% in elderly Japanese subjects aged ≥65 years. Several population-based studies have estimated the prevalence of

CMBs in elderly populations, with the resulting prevalence of CMBs in the elderly ranging from 11.1% to 34.4% (Table e-5).[9-13] These values were similar to the prevalence observed in our present study. The Sefuri Brain MRI Study, a population-based study for individuals aged ≥39 years who lived in a rural village in Japan, reported that the prevalence of CMBs in the Japanese elderly residents aged >60 years was 6.2%[7], which was lower than ours, probably because the former study excluded subjects with a history of neurological or psychiatric diseases, who were more likely to have CMBs than those without such a history. Some hospital- or institution-based studies in Japan[8,14,15,17] and Korea[16] have also reported lower prevalence of CMBs (3.1% to 6.8%) than the present study because these studies recruited healthy volunteers and included younger participants.

The previous pathological studies have shown that hypertensive angiopathy is mainly observed in deep or infratentorial brain areas, and amyloid angiopathy is mainly located in cerebral lobar regions.[2] Some population-based observational studies have reported that hypertension[5,8] or elevated blood pressure[8,9] was associated with deep/infratentorial CMBs. These findings were in accord with ours. Intriguingly, the present study found a significant association between hypertension and lobar CMBs. A pathological study failed to reveal a direct neuropathological association between amyloid angiopathy and lobar CMBs.[25] Another pathological study also found that amyloid angiopathy and fibrinoid necrosis, which is one of the major findings of hypertensive angiopathy, coexisted in lobar CMBs.[26] These findings raise the possibility that not only amyloid angiopathy, but also hypertensive angiopathy contributes to the development of lobar CMBs. Population-based observational studies in Western populations also showed that hypertensive subjects tended to have a higher risk of the presence of lobar CMBs than normotensive subjects, but the associations did not quite reach a statistically significant level.[5,9] The exact reason for this discrepancy in the influence

of hypertension on lobar vessels among populations is unknown, but it may be attributable to differences in the genetic backgrounds, age distributions,[5] and lifestyles (e.g., salt intake) among populations. Our study failed to show a clear association between blood pressure levels and lobar CMBs, probably because more than half of the participants were treated by antihypertensive agents.

In the present study, lower total cholesterol levels were associated with the presence of deep/infratentorial CMBs. Another study of Japanese individuals aged 27-95 years who underwent brain checkups with MRI also demonstrated a significant inverse association between serum total cholesterol levels and the likelihood of deep/infratentorial CMBs.[8] Lower serum total cholesterol levels might induce the formation of microaneurysms in the brain through fragility of the vessel walls, resulting in the occurrence of CMBs.[27] In support of this idea, a systematic review has shown that lower serum total cholesterol levels are significantly associated with hemorrhagic stroke.[28] In contrast, epidemiological studies conducted in Western populations found that lower cholesterol levels were associated with lobar CMBs, but not with deep/infratentorial CMBs.[5,9] The reason for the discrepancy between Japanese and Western studies is unclear but may be attributable to differences in the genetic backgrounds, the age distributions,[5] or the lower average levels of serum total cholesterol in the Japanese compared to the Western populations.[9]

In our subjects, APOE-ε4 was a significant risk factor for lobar CMBs but not for deep/infratentorial CMBs. The significant association of APOE-ε4 with lobar CMBs was also observed in the Framingham Heart Study[5] and the Rotterdam Study.[9] A meta-analysis[29] reported that APOE-ε4 was also significantly associated with cerebral amyloid angiopathy, which is considered to be one of the major causes of lobar CMBs. APOE-ε4 may induce

cerebral amyloid angiopathy by increasing β-amyloid aggregation, impairing β-amyloid clearance, or both.[30] On the other hand, the APOE-ε2 genotype was not associated with CMBs in the present study as well as in the previous reports.[9,10]

The community-based studies have reported inconsistent results in regard to the sex difference in CMB prevalence.[5-7,9-12] Some studies have reported that men had higher prevalence of CMBs than women[5,7,10,12] and others showed no sex difference.[6,9,11] In the present study, the age-adjusted model indicated that men had a higher risk of both deep/infratentorial and lobar CMBs than women. However, the sex difference in deep/infratentorial CMBs was diminished after adjustment for multiple risk factors, probably because men were likely to have more cardiovascular risk factors than women. In contrast, the sex difference in lobar CMBs was unchanged and remained significant after multivariable adjustment, indicating that residual confounding factors may exist in the association of male sex with a risk of lobar CMBs.

We showed that deep/infratentorial CMBs were more frequent than lobar CMBs. A study based on healthy volunteers in Japan also reported a similar finding.[8] In contrast, some Western studies reported higher prevalence of lobar CMBs than deep/infratentorial CMBs.[5,9-11] The reason for the discrepancy may be partially explained by the finding that Western people have a higher frequency of the APOE-ε4 genotype.[5,9,10]

The present study is the largest observational study to explore the prevalence of and risk factors for CMBs in a Japanese general elderly population. However, some limitations should be considered. First, although the participation rate of the present study was relatively high, approximately one third of the residents did not undergo MRI examination. The subjects

excluded from the study were likely to be older and to have more unhealthy backgrounds than those included in the study (Table e-6). Therefore, the prevalence of CMBs and the association between each risk factor and CMBs reported in this study might be underestimated. Second, because the present study was conducted in a cross-sectional manner, we cannot provide definite information about causal associations. Finally, we could not classify the cerebellar CMBs, which seemed to have different characteristics from other CMBs, because of limited sample size.

Conclusions

The present study suggests that approximately one of five elderly Japanese has some type of CMB and that subjects with CMBs have higher risk of symptomatic stroke and dementia. Deep/infratentorial CMBs and lobar CMBs have different risk factors, reflecting different pathological backgrounds. Further prospective studies are needed to elucidate risk factors for the development of CMBs and to establish strategies for prevention of CMBs and subsequent neurological disorders, including symptomatic stroke and dementia**.**

Table 1. Baseline characteristics of the subjects, the Hisayama Study, 2012

Variables	Total (n=1281)	Men (n=556)	Women (n=725)
Age, years	75 (7)	74 (6)	75 (7)
Systolic blood pressure, mmHg	134 (19)	135 (19)	134 (19)
Diastolic blood pressure, mmHg	76 (11)	78 (11)	75 (11)
Hypertension, %	70.7	71.6	70.0
Antihypertensive agent, %	55.8	57.1	54.8
Diabetes mellitus, %	23.3	30.2	17.9
Serum total cholesterol, mmol/l	5.11 (0.93)	4.80 (0.89)	5.35 (0.89)
BMI, kg/m^2	23.1 (3.3)	23.2 (3.0)	23.1 (3.6)
Current smoking, %	8.6	16.4	2.6
Current drinking, %	40.3	61.8	23.9
Regular exercise, %	19.1	21.2	17.5
Antithrombotic use, %	19.4	27.0	13.5
Antiplatelet use, %	15.5	22.1	10.5
Anticoagulant use, %	5.3	8.1	3.2
APOE-ε2 carrier, %	9.6	10.1	8.9
APOE-ε4 carrier, %	18.9	21.2	17.2
History of symptomatic stroke, %	5.3	7.6	3.6
Ischemic stroke, %	3.9	5.6	2.6
Intracerebral hemorrhage, %	1.1	1.8	0.6
Subarachnoid hemorrhage, %	0.3	0.2	0.4
Dementia, %	10.6	9.7	11.2
Lacunar infarction on MRI, %	22.2	30.1	16.1
WMH (Fazecas grade 2 or 3) on MRI, %	42.5	43.4	41.9

BMI, body mass index; APOE, apolipoprotein E.

Values are shown as means (standard deviation) or frequencies.

Table 2. Age-specific prevalence of any, deep/infratentorial, and lobar cerebral microbleeds, the Hisayama Study, 2012

	Number of participants	Any CMBs			Deep/infratentorial CMBs			Lobar CMBs		
		n	%	95% CI	n	%	95% CI	n	%	95% CI
Total										
Overall	1281	240	18.7	(16.6-20.8)	173	13.5	(11.6-15.4)	123	9.6	(8.0-11.2)
65-69 years	356	42	11.8	(8.4-15.2)	26	7.3	(5.0-10.0)	25	7.0	(4.3-9.7)
70-74 years	335	58	17.3	(13.2-21.4)	40	11.9	(8.4-15.4)	29	8.7	(5.7-11.7)
75-79 years	282	60	21.3	(16.5-26.1)	46	16.3	(12.0-20.6)	30	10.6	(7.0-14.1)
80-84 years	188	45	23.9	(17.8-30.0)	36	19.2	(13.6-24.8)	20	10.6	(6.2-15.0)
≥85 years	120	35	29.2	(21.1-37.3)	25	20.8	(13.5-28.1)	19	15.8	(9.3-22.3)
P for trend				<0.001			<0.001			0.001
Men										
Overall	556	128	23.0	(19.5-26.5)	90	17.4	(14.2-20.6)	71	12.8	(10.0-15.6)
65-69 years	153	30	19.6	(13.3-25.9)	20	13.1	(7.8-18.4)	15	9.8	(5.1-14.5)
70-74 years	148	25	16.9	(10.9-22.9)	15	10.1	(5.2-15.0)	17	11.5	(6.4-16.6)
75-79 years	128	32	25.0	(17.5-32.5)	27	21.1	(14.0-28.2)	15	11.7	(6.1-17.3)
80-84 years	78	23	29.5	(19.4-39.6)	17	21.8	(12.6-31.0)	11	14.1	(6.4-21.8)
≥85 years	49	18	36.7	(23.2-50.2)	11	22.5	(10.8-34.2)	13	26.5	(14.1-38.9)
P for trend				0.003			0.01			0.01
Women										
Overall	725	112	15.5	(12.9-18.1)	83	11.9	(9.5-14.3)	52	7.2	(5.3-9.1)
65-69 years	203	12	5.9	(2.7-9.1)	6	3.0	(0.7-5.3)	10	4.9	(1.9-7.9)
70-74 years	187	33	17.7	(12.2-23.2)	25	13.4	(8.5-18.3)	12	6.4	(2.9-9.9)
75-79 years	154	28	18.2	(12.1-24.3)	19	12.3	(7.1-17.5)	15	9.7	(5.0-14.4)
80-84 years	110	22	20.0	(12.5-27.5)	19	17.3	(10.2-24.4)	9	8.2	(3.1-13.3)
≥85 years	71	17	23.9	(14.0-33.8)	14	19.7	(10.4-29.0)	6	8.5	(2.0-15.0)
P for trend				<0.001			<0.001			0.07

CMBs, cerebral microbleeds; CI, confidence interval.

Table 3. Association of risk factors with the presence of any cerebral microbleeds

Variables	Age- and sex-adjusted			Multivariable-adjusted		
	OR	95% CI	*P*	OR	95% CI	*P*
Age (per 10 year increase)	1.65	(1.35-2.03)	<0.001	1.47	(1.16-1.87)	0.002
Men	1.70	(1.28-2.26)	<0.001	1.39	(0.97-2.00)	0.08
Hypertension	2.47	(1.68-3.62)	<0.001	2.19	(1.44-3.33)	<0.001
Diabetes mellitus	1.29	(0.93-1.79)	0.13	1.08	(0.75-1.54)	0.69
Serum total cholesterol (per 1 mmol/l increase)	0.89	(0.75-1.05)	0.15	0.89	(0.74-1.07)	0.22
BMI (per 1 kg/m^2 increase)	1.03	(0.99-1.08)	0.13	0.99	(0.95-1.04)	0.80
Current smoking	0.81	(0.48-1.39)	0.45	1.13	(0.63-2.03)	0.67
Current drinking	1.18	(0.86-1.63)	0.31	1.10	(0.77-1.56)	0.60
Regular exercise	0.86	(0.59-1.25)	0.42	0.84	(0.56-1.27)	0.41
Antithrombotic treatment	1.57	(1.12-2.21)	0.01	1.20	(0.82-1.75)	0.35
APOE-ε2 carrier	0.89	(0.53-1.51)	0.67	0.94	(0.55-1.61)	0.82
APOE-ε4 carrier	1.46	(1.02-2.10)	0.04	1.42	(0.98-2.06)	0.06

OR, odds ratio; CI, confidence interval; BMI, body mass index; APOE, apolipoprotein E.

Table 4: Association of risk factors with the presence of deep/infratentorial cerebral microbleeds

	Age and sex-adjusted			Multivariable-adjusted		
Variables	OR	95%CI	*P*	OR	95%CI	*P*
Age (per 10 year increase)	1.75	(1.39-2.22)	<0.001	1.49	(1.14-1.95)	0.004
Men	1.63	(1.18-2.26)	0.003	1.20	(0.79-1.82)	0.39
Hypertension	2.67	(1.69-4.21)	<0.001	2.20	(1.35-3.60)	0.002
Diabetes mellitus	1.56	(1.09-2.24)	0.02	1.37	(0.92-2.02)	0.12
Serum total cholesterol (per 1 mmol/l increase)	0.76	(0.63-0.93)	0.01	0.78	(0.63-0.97)	0.03
BMI (per 1 kg/m^2 increase)	1.04	(0.99-1.09)	0.15	0.99	(0.94-1.05)	0.75
Current smoking	0.93	(0.51-1.68)	0.80	1.25	(0.65-2.38)	0.51
Current drinking	1.18	(0.82-1.70)	0.38	1.13	(0.75-1.68)	0.56
Regular exercise	0.83	(0.53-1.29)	0.40	0.84	(0.53-1.34)	0.46
Antithrombotic treatment	1.65	(1.13-2.41)	0.01	1.19	(0.78-1.82)	0.41
APOE-ε2 carrier	0.83	(0.45-1.53)	0.54	0.79	(0.42-1.49)	0.47
APOE-ε4 carrier	1.04	(0.67-1.62)	0.88	0.99	(0.63-1.57)	0.97

OR, odds ratio; CI, confidence interval; BMI, body mass index; APOE, apolipoprotein E.

Table 5. Association of risk factors with the presence of lobar cerebral microbleeds

Variables	Age-sex-adjusted			Multivariable-adjusted		
	OR	95%CI	*P*	OR	95%CI	*P*
Age (per 10 year increase)	1.58	(1.21-2.07)	<0.001	1.40	(1.01-1.93)	0.04
Men	2.00	(1.37-2.93)	<0.001	1.82	(1.12-2.97)	0.02
Hypertension	2.11	(1.28-3.47)	0.003	1.89	(1.09-3.28)	0.02
Diabetes mellitus	0.94	(0.59-1.48)	0.78	0.69	(0.41-1.17)	0.17
Serum total cholesterol (per 1 mmol/l increase)	0.92	(0.73-1.15)	0.44	0.93	(0.72-1.19)	0.54
BMI (per 1 kg/m^2 increase)	1.03	(0.97-1.09)	0.36	1.00	(0.94-1.07)	0.99
Current smoking	0.70	(0.34-1.45)	0.34	1.03	(0.48-2.23)	0.93
Current drinking	1.11	(0.72-1.69)	0.64	0.99	(0.62-1.60)	0.99
Regular exercise	0.89	(0.54-1.46)	0.61	0.87	(0.51-1.51)	0.63
Antithrombotic treatment	1.87	(1.22-2.88)	0.004	1.48	(0.90-2.41)	0.12
APOE-ε2 carrier	1.05	(0.54-2.06)	0.88	1.16	(0.59-2.31)	0.66
APOE-ε4 carrier	1.73	(1.09-2.74)	0.02	1.70	(1.08-2.80)	0.02

OR, odds ratio; CI, confidence interval; BMI, body mass index; APOE, apolipoprotein E.

References

1. Offenbacher H, Fazekas F, Schmidt R, Koch M, Fazekas G, Kapeller P. MR of cerebral abnormalities concomitant with primary intracerebral hematomas. *Am J Neuroradiol*. 1996;17:573-578.
2. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain*. 2007;130:1988-2003.
3. Ioannidis JP, Ntzani EE, Trikalinos TA. 'Racial' differences in genetic effects for complex diseases. *Nat Genet.* 2004;36:1312-1318.
4. Hata J, Kiyohara Y. Epidemiology of stroke and coronary artery disease in Asia. *Circulation J.* 2013:77:1923-1932.
5. Romero JR, Preis SR, Beiser A, et al. Risk factors, stroke prevention treatments, and prevalence of cerebral microbleeds in the Framingham Heart Study. *Stroke*. 2014;45:1492-1494.
6. Roob G, Schmidt R, Kapeller P, Lechner A, Hartung HP, Fazekas F. MRI evidence of past cerebral microbleeds in a healthy elderly population. *Neurology*. 1999;52:991-994.
7. 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.
8. Mitaki S. Nagi A, Oguro H, Yamaguchi S. Serum lipid fractions and cerebral microbleeds in a healthy Japanese population. *Cerebrovasc Dis*. 2017;43:186-191.
9. Vernooij MW, van der Lugt A, Ikram MA, et al. Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. *Neurology*. 2008;70:1208-1214.
10. 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.

11. Wiegman AF, Meier IB, Schupf N, et al. Cerebral microbleeds in a multiethnic elderly community: demographic and clinical correlates. *J Neurol Sci*. 2014;345:125-130.
12. Gustavsson AM, Stomrud E, Abul-Kasim K, et al. Cerebral microbleeds and white matter hyperintensities in cognitively healthy elderly: a cross-sectional cohort study evaluating the effect of arterial stiffness. *Cerebrovasc Dis Extra*. 2015;5:41-51.
13. Hilal S, Mok V, Youn YC, Wong A, Ikram MK, Chen CL. Prevalence, risk factors and consequences of cerebral small vessel diseases: data from three Asian countries. *J Neurol Neurosurg Psychiatry.* 2017;88:669-674.
14. Igase M, Tabara Y, Igase K, et al. Asymptomatic cerebral microbleeds seen in healthy subjects have a strong association with asymptomatic lacunar infarction. *Circ J.* 2009; 73**:** 530-533.
15. Tsushima Y, Tanizaki Y, Aoki J, Endo K. MR detection of microhemorrhages in neurologically healthy adults. *Neuroradiology.* 2002;44:31-36.
16. Jang SH, Lee H, Kim JS, et al. Association between helicobacter pylori infection and cerebral small vessel disease. *Korean J Fam Med*. 2015;36:227-232.
17. Yakushiji Y, Nishiyama M, Yakushiji S, et al. Brain microbleeds and global cognitive function in adults without neurological disorder. *Stroke*. 2008;39:3323-3328.
18. Hata J, Ninomiya T, Hirakawa Y, et al. Secular trends in cardiovascular disease and its risk factors in Japanese half-century data from the Hisayama Study (1961–2009). *Circulation*. 2013;128:1198-1205.
19. Hirabayashi N, Hata J, Ohara T, et al. Association between diabetes and hippocampal atrophy in elderly Japanese: the Hisayama Study. *Diabetes Care*. 2016;39:1543-1549.
20. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *Am J Roentgenol* .1987;149:351-356.

21. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. *J Hum Genet*. 2001;46:471-477.
22. Ohara T, Ninomiya T, Kubo M, et al. Apolipoprotein genotype for prediction of Alzheimer's disease in older Japanese: the Hisayama Study. *J Am Geriatr Soc*. 2011;59:1074-1079.
23. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 3rd Ed. Revised. Washington, DC: American Psychiatric Association; 1987.
24. Ohara T, Hata J, Yoshida D, et al. Trends in dementia prevalence, incidence, and survival rate in a Japanese community. *Neurology* 2017;16:1925-1932.
25. Kövari E, Charidimou A, Herrmann FR, Giannakopoulos P, Bouras C, Gold G. No neuropathological evidence for a direct topographical relation between microbleeds and cerebral amyloid angiopathy. *Acta Neuropathol Commun*. 2015;3:49.
26. van Veluw SJ, Biessels GJ, Klijn CJ, Rozemuller AJ. Heterogeneous histopathology of cortical microbleeds in cerebral amyloid angiopathy. *Neurology*. 2016;86:867-871.
27. Konishi M, Iso H, Komachi Y, et al. Associations of serum total cholesterol, different types of stroke, and stenosis distribution of cerebral arteries the Akita Pathology Study. *Stroke*. 1993;24:954-964.
28. Wang X, Dong Y, Qi X, Haung C, Hou L. Cholesterol levels and risk of hemorrhagic stroke: a systematic review and meta-analysis. *Stroke*. 2013;44:1833-1839.
29. Rannikmae K, Samarasekera N, Martinez-Gonzalez NA, Al-Shahi Salman R, Sudlow CL. Genetics of cerebral amyloid angiopathy: systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry*. 2013;84:901-908.
30. Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer's disease and other neurological disorders. *Lancet Neurol*. 2011;10:241-252.

Supplemental data

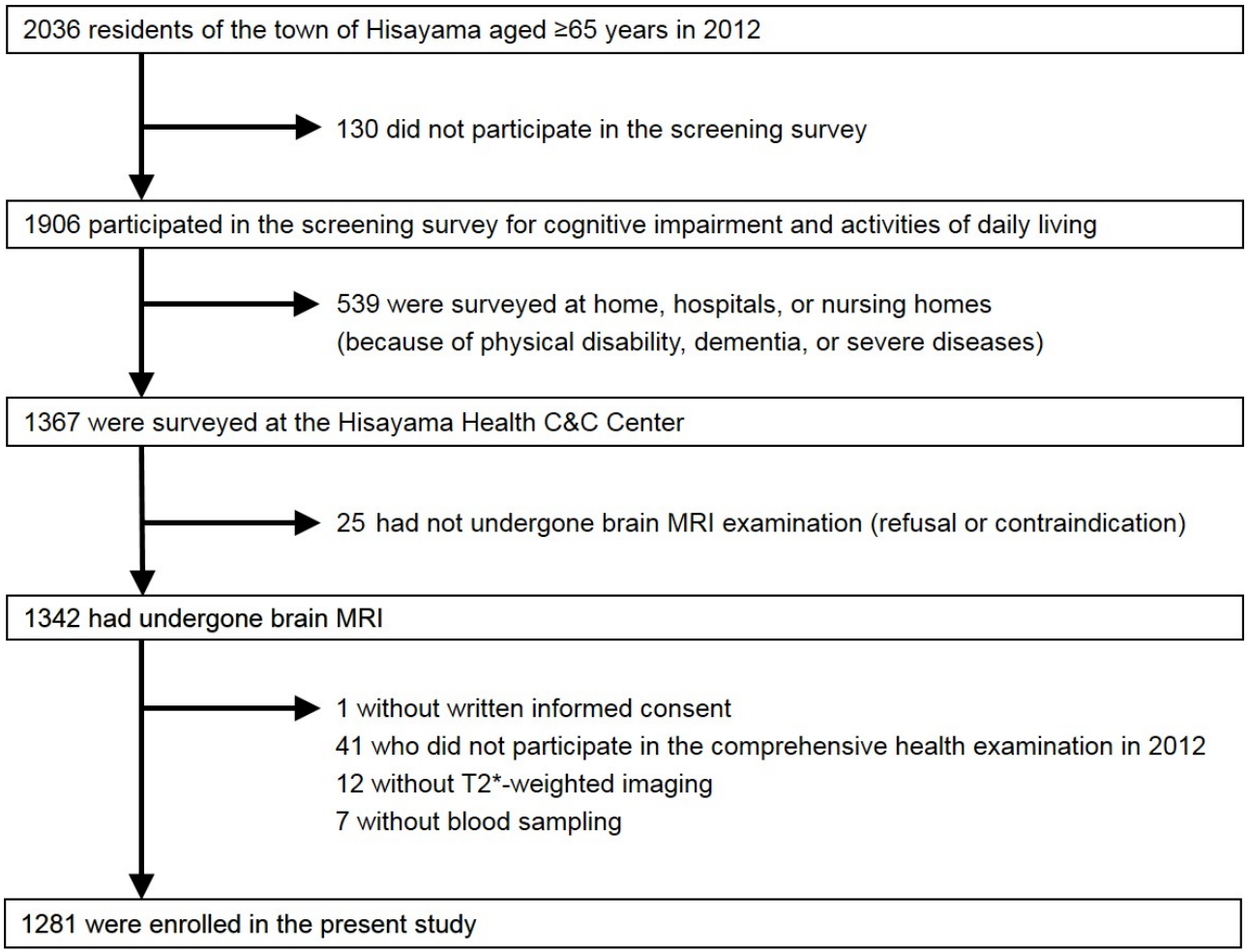

Figure e-1. Selection of study participants

A total of 1906 individuals participated in a screening survey for cognitive impairment and activities of daily living in 2012. The present study included 1342 participants with T2*-weighted imaging of MRI and data for cardiovascular risk factors.

Table e-1. Association of risk factors with the presence of strictly lobar cerebral microbleeds

	Age-sex-adjusted			Multivariable-adjusted		
Variables	OR	95%CI	*P*	OR	95%CI	*P*
Age (per 10 year increase)	1.48	(1.02-2.15)	0.04	1.41	(0.91-2.20)	0.13
Men	1.96	(1.15-3.34)	0.01	2.13	(1.11-4.11)	0.02
Hypertension	2.09	(1.03-4.24)	0.04	2.12	(1.00-4.50)	0.04
Diabetes mellitus	0.49	(0.23-1.06)	0.08	0.42	(0.18-0.97)	0.04
Serum total cholesterol (per 1 mmol/l increase)	1.33	(0.97-1.82)	0.08	1.30	(0.93-1.83)	0.12
BMI (per 1 kg/m^2 increase)	0.99	(0.91-1.07)	0.77	0.99	(0.91-1.09)	0.90
Current smoking	0.84	(0.29-2.47)	0.75	0.91	(0.30-2.74)	0.87
Current drinking	1.20	(0.66-2.19)	0.55	1.03	(0.55-1.93)	0.92
Regular exercise	0.78	(0.38-1.64)	0.52	0.80	(0.38-1.71)	0.57
Antithrombotic treatment	1.32	(0.70-2.49)	0.39	1.21	(0.60-2.45)	0.59
APOE-ε2 carrier	1.05	(0.44-2.53)	0.91	1.44	(0.58-3.59)	0.44
APOE-ε4 carrier	2.95	(1.69-5.14)	<0.001	2.97	(1.66-5.34)	<0.001

OR, odds ratio; CI, confidence interval; BMI, body mass index; APOE, apolipoprotein E.

Strictly lobar cerebral microbleeds (CMBs) were defined as lobar CMBs without deep/infratentorial CMBs.

Table e-2. Prevalence of CMBs by APOE-ε4 status

	APOE-ε4 genotype		
	Carrier (n=209)	Non-carrier (n=897)	*P*
Any CMBs, %	24.9	18.5	0.04
Deep/infratentorial CMBs, %	15.6	15.1	0.95
Lobar CMBs, %	16.0	9.9	0.02

CMBs, cerebral microbleeds; APOE, apolipoprotein E.

Table e-3. Association of blood pressure levels and antihypertensive treatment status with the presence of cerebral microbleeds

	Any CMBs			Deep/infratentorial CMBs			Lobar CMBs		
Variables	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Blood pressure levels									
<140/90 mmHg	1.00	(reference)		1.00	(reference)		1.00	(reference)	
140-159/90-99 mmHg	1.10	(0.78-1.55)	0.59	1.15	(0.77-1.70)	0.50	1.17	(0.74-1.83)	0.50
≥160/100 mmHg	1.45	(0.85-2.47)	0.17	1.84	(1.04-3.26)	0.03	0.83	(0.35-1.94)	0.60
Antihypertensive treatment									
No	1.00	(reference)		1.00	(reference)		1.00	(reference)	
Yes	2.09	(1.44-3.01)	<0.001	2.01	(1.31-3.07)	0.001	2.14	(1.30-3.54)	0.03

CMBs, microbleeds; OR, odds ratio; CI, confidence interval.

OR (95% CI) was estimated using the multivariable logistic regression model including blood pressure levels (3 categories), antihypertensive treatment status, and other cardiovascular risk factors (age, sex, hypertension, diabetes, serum total cholesterol, body mass index, current smoking, current drinking, regular exercise, use of antithrombotic agents, and APOE polymorphisms (ε2 and ε4)).

Table e-4. Association of CMBs with other neurological disorders

	History of symptomatic ischemic stroke			History of symptomatic intracerebral hemorrhage			Dementia		
Variables	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Any CMBs	2.85	(1.58-5.15)	<0.001	29.16	(5.36-113.80)	<0.001	2.51	(1.65-3.83)	<0.001
Deep/infratentorial CMBs	2.74	(1.40-5.37)	0.003	43.82	(9.45-203.10)	<0.001	2.87	(1.81-4.54)	<0.001
Lobar CMBs	2.76	(1.32-5.79)	0.01	34.56	(6.89-173.28)	<0.001	3.77	(2.24-6.33)	<0.001

	Lacunar infarction on MRI			White matter hyperintrensity (Fazecas grade 2 or 3) on MRI		
Variables	OR	95% CI	*P*	OR	95% CI	*P*
Any CMBs	3.24	(2.37-4.42)	<0.001	2.35	(1.73-3.17)	<0.001
Deep/infratentorial CMBs	4.37	(1.38-2.13)	<0.001	2.74	(1.93-3.90)	<0.001
Lobar CMBs	3.01	(2.00-4.54)	<0.001	3.11	(2.05-4.72)	<0.001

CMBs, cerebral microbleeds; OR, odds ratio; CI, confidence interval.

OR (95% CI) was adjusted for age and sex.

Table e-5. Prevalence of CMBs among the elderly subjects in the previous population-based studies and the present study

Study	Country	Study period	Age (year)	Prevalence of CMBs (%)
Rotterdam Study[9]	Netherlands	2005-2006	≥60	23.5
AGES-Reykjavik Study[10]	Iceland	2002-2004	67-92	11.1
Washington Heights/Inwood Columbia Aging Project[11]	United States	2005-2007	≥65	27.6
Swedish BioFinder Study[12]	Sweden	2009-2013	>60	12.1
EDIS Study[13]	Singapore	2010-2015	≥60	34.4
RISK Study[13]	Hong Kong	2011-2015	≥65	21.1
Hisayama Study (the present study)	Japan	2012	65-97	18.7

CMBs, cerebral microbleeds; AGES, Age, Gene/Environment Susceptibility; EDIS, Epidemiology of Dementia In Singapore; RISK, Risk Index for Subclinical brain lesions in Hong Kong.

Table e-6. Baseline characteristics of the subjects included in and excluded from the present study

Variables	Included (n=1281)	Excluded * (n=323)	*P*
Age, years	74 ± 7	81 ± 9	<0.001
Men, %	43.4	33.8	0.002
Systolic blood pressure, mmHg	70.7	76	0.08
Diastolic blood pressure, mmHg	134 ± 19	140 ± 24	<0.001
Hypertension, %	76 ± 11	76 ± 13	0.54
Antihypertensive agent, %	55.6	55.6	1.00
Diabetes mellitus, %	23.3	22.2	0.99
Serum total cholesterol, mmol/l	5.12 (0.93)	4.86 (0.98)	<0.001
BMI, kg/m^2	23.1 (3.3)	21.8 (3.9)	<0.001
Current smoking, %	8.6	5.0	0.04
Current drinking, %	40.3	21.1	<0.001
Regular exercise, %	19.1	5.3	<0.001
Antithrombotic use, %	19.4	31.6	<0.001
Antiplatelet use, %	15.5	23.8	<0.001
Anticoagulant use, %	5.3	10.8	<0.001
APOE-ε2 carrier, %	9.6	9.5	1.00
APOE-ε4 carrier, %	18.9	26.3	0.01
History of symptomatic stroke, %	5.3	16.4	<0.001
Ischemic stroke, %	3.9	10.2	<0.001
Intracerebral hemorrhage, %	1.1	4.0	<0.001
Subarachnoid hemorrhage, %	0.3	2.2	0.001
Dementia, %	10.6	45.3	<0.001

BMI, body mass index; APOE, apolipoprotein E.

Values are shown as means (standard deviation) or frequencies.

*Baseline characteristics data were available for 323 of the 625 residents excluded from the study.