ORIGINAL RESEARCH

The Prevalence and Risk Factors of Cerebral Microbleeds: A Community-Based Study in China

Qi Luo [1,*]
Huidong Tang [1,*]
Xinxin Xu [2]
Juan Huang [2]
Pei Wang [1]
Guiying He [1]
Xiaoxuan Song [1]
Yumeng Huang [1]
Shengdi Chen [1]
Fuhua Yan [2]
Yuyan Tan [1]
Jianfang Ma [1]

[1]Department of Neurology, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China; [2]Department of Radiology, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

*These authors contributed equally to this work

Introduction: Cerebral microbleeds (CMBs) are frequently found in the healthy elderly. However, data on the prevalence and risk factors of CMBs in the general population of China are lacking.

Methods: A cross-sectional study focusing on the prevalence and risk factors of CMBs was conducted in stroke-free elderly from Shanghai Wuliqiao community. MRI was performed at 3Tesla and cardiovascular risk factors (eg, age, smoking history, and hypertension), cerebral small vessel disease (CSVD) markers (eg, white matter hyperintensities, lacunar infarction, and enlarged perivascular space) and genetic information (eg, APOE, CR1) were recorded. Poisson regression was used to analyze the risk factors of the presence and location of microbleeds.

Results: A total of 199 participants (70.8±7.2 years old; male 31.2%) were finally included in our analysis. The overall prevalence of CMBs was 12.6% (25/199) and increased with age from 7.5% (55–64 years old) to 19.3% (over 75 years old). Of those with CMBs, most of them (16/25) located in the deep/mixed region and had 1–2 CMBs (18/25). Poisson regression analysis showed that white matter hyperintensities (OR=1.22, 95% CI: 1.16–1.29), APOE ε4+ carrier (OR=2.16, 95% CI: 1.18–3.96) and CR1 non-F/F isoform (OR=7.78, 95% CI: 4.34–13.96) were associated with CMBs. Further analysis found that in addition to the above three risk factors, hypertension (OR=2.98, 95% CI: 1.16–7.64), lacunar infarction (OR=2.39, 95% CI: 1.19–4.81) also increased the risk of deep/mixed CMBs.

Conclusion: The prevalence of cerebral microbleeds is similar to other countries. Cardiovascular risk factors, CSVD markers, and genetic factors (APOE ε4, CR1 non-F/F isoform) were associated with CMBs, suggesting an interaction of multiple pathogenesis in Chinese stroke-free community population.

Keywords: cerebral microbleeds, prevalence, risk factors, community-based study

Introduction

Cerebral microbleeds (CMBs) are 2–10mm, rounded or circular, well-defined hypointense lesions on gradient-echo T2*-weighted images (GRE T2*WI) or susceptibility-weighted images (SWI) of magnetic resonance imaging (MRI).[1] As one feature of cerebral small vessel disease (CSVD), CMBs were found to have a close association with ischemic or hemorrhagic stroke, dementia, or even mortality.[2]

The prevalence of CMBs in the normal elderly varies across different studies, ranging from 4.0%[3] to 26.9%.[4] However, limited studies have been done in China. There was one study done in Shanghai urban area that reported a prevalence of 7.3%,[5] but with 1.5T scanner, which may reduce the detection of CMBs. Another study was done on rural areas and showed the prevalence of 10.6%.[6]

Correspondence: Jianfang Ma
Department of Neurology, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, No. 197, Second Ruijin Road, Shanghai, 200025, People's Republic of China
Tel/Fax +86 21-6445-4473
Email mjf10924@rjh.com.cn

The etiology of CMBs is complex. Many studies suggested that deep CMBs may relate to hypertensive small vessel disease (HTN-SVD) and strictly lobar CMBs for cerebral amyloid angiopathy (CAA),[1] but the risk factors of CMBs are not entirely clear. Previous studies found APOE genotype, cardiovascular factors, and CSVD were associated with CMBs.[7] With the different distribution of CMBs between Western and Eastern countries,[3] it remains unknown whether there are also different risk factors of CMBs either. Studies in Japan found hypertension and lower serum total cholesterol were associated with deep/infratentorial CMBs risk, while APOE ε4 was related to lobar CMBs.[8] Other Asian countries like Singapore reported age and hypertension were risk factors of CSVD.[4] Few studies focused on the risk factors of CMBs in Chinese population. Only one study of 1211 individuals in Shunyi city confirmed age and hypertension increased the risk of deep CMBs. However, this study did not have CSVD evaluation and genetic investigation.[6]

Hence, we performed a cross-sectional study with 3T MRI to investigate the prevalence and risk factors of CMBs, including cardiovascular factors (eg, hypertension, diabetes), CSVD evaluation (eg, white matter hyperintensity, lacunar infarction, and enlarged perivascular space) and genetic investigation (eg, APOE, CR1), in a stroke-free Chinese community cohort.

Method
Study Participants
The Wuliqiao cohort is an ongoing longitudinal community-based study designed to assess risk factors of neurodegenerative diseases including dementia and Parkinson's disease. The inclusion criteria for Wuliqiao cohort: A) aged ≥50 years; B) willing to participate. Three thousand nine hundred and seventy inhabitants were recruited in Wuliqiao community (urban) of Shanghai at baseline (2009–2011) and followed up in 2014–2015 and 2018–2019.[9,10]

Participants in this study (MRI sub-study) were enrolled during the second follow-up 2018–2019 period. Inclusion criteria included: A) willing to participate; B) no MRI contraindications: implantable devices, intraorbital metallic foreign body, intracranial aneurysm clips, cardiac pacemaker, valvular prosthesis, and cochlear implants; C) free of stroke: individuals without a history of symptomatic ischemic stroke and intracerebral hemorrhage (ICH), which should be confirmed by CT or MRI; D) free of other

neurological diseases including major head trauma, brain surgery, brain tumor, psychiatric diseases, Parkinson's disease and dementia. Two hundred and nine individuals were enrolled in which 10 individuals were excluded due to claustrophobia (n=6) and poor image pictures (n=4). Therefore, a total of 199 individuals with eligible data were included in the final analysis.

Standard Protocol Approval, Registration, and Patient Consent
The study was approved by Ethics Committee of Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, China. Written informed consent was obtained from each participant of this study.

Cardiovascular Risk Factors
Cardiovascular risk factors were collected at the time of MRI by interview, laboratory, or physical examination. Demographic information including age at MRI, sex, height and weight, and smoking history were documented. Related diseases, including hypertension, diabetes, and atrial fibrillation, and medicine history, including antithrombotics (antiplatelets and anticoagulants) and statins, were also documented (See supplemental data).

Magnetic Resonance Imaging Parameters and CSVD Evaluations
All subjects were scanned on 3T Philips Ingenia (Philips Healthcare, Best, The Netherlands) at the Radiology Department of Ruijin Hospital. Susceptibility-weighted images (SWI; repetition time (TR) =25ms, echo time (TE) =18ms, field of view (FOV) = 256×256 mm^2, 54 slices, 2-mm slice thickness) were acquired in axial plane. Three-dimensional (3D) T1-weighted images (T1WI; TE=7.5ms, TR=25ms, 64 slices, 2-mm slice thickness) and fluid-attenuated inversion recovery, spectral presaturation with inversion recovery (Flair-SPIR; TE=120ms, TR=9000ms, 32 slices, 3-mm slice thickness) were also acquired. All CSVD evaluation (including CMBs) was done by two experienced radiologists (See supplemental data).

Microbleed anatomical rating scale (MARS) was used to evaluate the number and location of CMBs.[11] Lobar region includes cortical and subcortical regions with U fibers. Basal ganglia, thalamus, internal capsule, external capsule, corpus callosum, and deep and periventricular white matter belong to the deep region, and the

infratentorial region is composed of brainstem and cerebellum. Scale of age-related white matter changes (ARWMC) was used as a general measure of white matter hyperintensities.[12] White matter changes on MRI were defined as bright lesions ≥5 mm on FLAIR images. Lacune was rated visually as a deep, focal lesion (3–15 mm), hypointense in 3D T1WI and had a hyperintense rim surrounding the lesions in FLAIR sequence.[13] Enlarged perivascular space (EPVS) was defined as visible small dots or linear structures of hypointensity (≤3 mm) in 3D T1WI and FLAIR.[14] EPVS was evaluated in basal ganglia and centrum semiovale, respectively.

Apolipoprotein E Genotyping and CR1 Isoforms Test

Apolipoprotein E (APOE) genotyping was performed by company (MAP Biotech, Shanghai). APOE genotyping was available for 196 participants (98.5%). The genotype and allele frequencies of rs429358 and rs7412 in our population were in Hardy–Weinberg equilibrium.

Complement C3b/C4b receptor 1 (CR1) isoforms were determined by Western blot and paralogue ratio test (PRT) tests (See supplemental data). The different isoforms of CR1 reflect the copy number variations, and F isoform (190kDa) reflects 2 copy numbers, S isoform (220kDa) reflects 3 copy numbers and the rare F' isoform (160kDa) with 1 copy number.[15] Most individuals carried F/F isoform and few CR1 non-F/F isoform, including 11 F/S isoform, 2 S/S isoform, and 3 F/F' isoform (See supplemental data).

Statistics

Of those with CMBs, two groups were divided according to the previous studies.[7] One was individuals with CMBs restricted to a lobar region, called strictly lobar CMBs, and the other was individuals with CMBs in a deep region, with or without lobar CMBs, called deep/mixed CMBs. Distribution of cerebellar microbleed may be related with different pathogenic mechanism,[16] and we did not include 1 individual with strictly cerebellar microbleed into the group of deep/mixed CMBs. CR1 was divided into two groups, including F/F isoform and non-F/F isoform (F/S, S/S, F/F' isoform). Poisson regression, which makes full use of the information about the number of CMBs, was used to analyze the risk factors of CMBs. Model 1 adjusted for age and sex and Model 2 adjusted for all positive factors in Model 1. Categorical variables are presented as count (percentage, %) and continuous variables as mean (standard deviation, SD) or median (interquartile range, IQR). P value <0.05 was considered statistically significant. All analyses were performed by SPSS 25.0.

Results

Total of 199 participants were included in this study. Mean age was 70.8 years old and 62 (31.2%) were male. The demographic data on brain MRI variables and genetic information were shown in Table 1. The prevalence of overall CMBs was 12.6% and increased along with age (Table 2). Of those with CMBs (n =25), 8 individuals had strictly lobar CMBs (only 2 with multiple (n≥2) strictly lobar CMBs), 16 had deep/mixed CMBs (8 strictly deep CMBs and 8 CMBs in both lobar and deep regions), and 1 had strictly infratentorial CMBs.

We conducted Poisson regression analysis to investigate the risk factors of CMBs (Table 3). Due to the limited sample size, analyses were performed in all CMBs and deep/mixed CMBs, but not strictly lobar CMBs. APOE ε4 + carriers (OR=2.16, 95% CI: 1.18–3.96), CR1 non-F/F isoform (OR=7.78, 95% CI: 4.34–13.96) and white matter hyperintensities (OR=1.22, 95% CI: 1.16–1.29) increased the risk of all CMBs. However, diabetes (OR=0.24, 95%

Table 1 Demographic and Clinical Characteristics of Participants

	All (n=199)
Age at MRI, y, mean (SD)	70.8 (7.2)
Sex, male, n (%)	62 (31.2)
Obesity [a], n (%)	18 (9.0)
Smoking history, n (%)	36 (18.1)
Hypertension, n (%)	91 (45.7)
Diabetes, n (%)	28 (14.1)
Atrial fibrillation, n (%)	5 (2.5)
Antithrombotics, n (%)	70 (35.2)
Statin, n(%)	32 (16.1)
Microbleeds, n(%)	25 (12.6)
ARWMC, Median (IQR) [b]	5 (3,8)
Lacunar Infarction, n (%) [b]	33 (16.8)
EPVS, n (%) [b,c]	16 (8.1)
APOE ε4 + carrier, n (%) [d]	39 (19.9)
CR1 non-F/F isoform, n (%) [e]	16 (8.0)

Notes: [a]Obesity was considered when BMI ≥ 28kg/m[2;] [b]Two individuals fail to complete the FLAIR sequence; [c]15 individuals with basal ganglion-EPVS predominated and 1 individuals with equal degree; [d]Three individuals did not give consent for genotyping; [e]CR1 non-F/F isoform includes 11 F/S isoform, 2 S/S isoform, and 3 F/F' isoform.
Abbreviations: CMBs, cerebral microbleeds; MRI, magnetic resonance imaging; ARWMC, age-related white matter changes; EPVS, enlarged perivascular space; APOE, apolipoprotein E; CR1, complement C3b/C4b receptor 1; SD, standard deviation; IQR, interquartile range.

Table 2 Age-Specific Prevalence of CMBs

Age Range	Participants, n	CMB, % (n)	Multiple (n≥2) CMBs, % (n)	Strictly Lobar CMBs, % (n)
55–64 y	40	7.5 (3)	2.5 (1)	2.5 (1)
65–74 y	102	10.8 (11)	4.9 (5)	3.9 (4)
75–92 y	57	19.3 (11)	10.5 (6)	5.3 (3)

Abbreviation: CMBs, cerebral microbleeds.

Table 3 Risk Factors of CMBs by Poisson Regression

Variables	Overall CMB vs No CMB, OR (95% CI)[h]		Deep/Mixed vs No CMB, OR (95% CI)	
	Model 1	Model 2	Model 1	Model 2
Age [a], per year	**1.07 (1.03,1.10)**	1.02 (0.98,1.06)	**1.06 (1.02,1.09)**	0.99 (0.93,1.05)
Sex [b], male, vs female	**2.16 (1.39,3.38)**	0.83 (0.33,2.05)	**3.39 (2.00,5.76)**	1.17 (0.37,3.70)
Obesity [c]	**3.24 (1.96,5.34)**	1.11 (0.52,2.40)	**4.45 (2.61,7.61)**	0.70 (0.26,1.93)
Smoking history	**3.80 (1.96,7.40)**	1.43 (0.55,3.70)	**4.66 (2.22,9.78)**	0.99 (0.29,3.40)
Hypertension	**2.18 (1.35,3.24)**	1.64 (0.92,2.92)	**4.52 (2.35,8.69)**	**2.98 (1.16,7.64)**
Diabetes	**0.17 (0.05,0.55)**	**0.24 (0.07,0.77)**	**0.14 (0.03,0.57)**	0.23 (0.05,1.06)
Atrial fibrillation	2.02 (0.63,6.49)	–	2.81 (0.86,9.16)	-
Antithrombotics	**0.39 (0.23,0.68)**	0.69 (0.38,1.26)	**0.49 (0.27,0.89)**	1.07 (0.51,2.24)
Statin	0.85 (0.47,1.56)	–	1.19 (0.63,2.23)	-
ARWMC [d]	**1.25 (1.19,1.31)**	**1.22 (1.16,1.29)**	**1.33 (1.26,1.41)**	**1.38 (1.27,1.49)**
Lacunar Infarction [d]	**1.64 (1.00,2.71)**	1.29 (0.76,2.19)	**2.11 (1.22,3.64)**	**2.39 (1.19,4.81)**
EPVS [d,e]	1.57 (0.81,3.05)	–	1.91 (0.94,3.87)	–
APOE ε4+ carrier [f]	**3.16 (2.00,4.99)**	**2.16 (1.18,3.96)**	**4.33 (2.60,7.20)**	**3.72 (1.60,8.65)**
CR1 non-F/F [g]	**7.26 (4.62,11.42)**	**7.78 (4.34,13.96)**	**10.85 (6.47,18.21)**	**23.97 (10.56,54.42)**

Notes: Model 1: adjusted for age and sex. Model 2: adjusted for all positive predictors in Model 1. Bold text represents statistically significant (p<0.05); [a]adjusted for sex, [b]adjusted for age; [c]Obesity was considered when BMI ≥ 28kg/m²; [d]Two individuals fail to complete the FLAIR sequence; [e]15 individuals with basal ganglion-EPVS predominated and 1 individuals with equal degree. [f]Three individuals did not give consent for genotyping; [g]CR1 non-F/F isoform includes 11 F/S isoform, 2 S/S isoform, and 3 F/F' isoform; [h]Further analyses on Strictly lobar vs no CMB was ceased due to poor goodness-of-fit.
Abbreviations: CMBs, cerebral microbleeds; MRI, magnetic resonance imaging; ARWMC, age-related white matter changes; EPVS, enlarged perivascular space; APOE, apolipoprotein E; CR1, complement C3b/C4b receptor 1; OR, odds ratio; 95% CI, 95% confidence interval.

CI: 0.07–0.77) reduced the risk of all CMBs in Model 2. Other risk factors including age, male sex, obesity, smoking history, hypertension, antithrombotics, and lacunar infarction found in Model 1 did not remain statistically significant in Model 2.

Risk factors for deep/mixed CMBs were partially similar to the results of overall CMBs. The difference was that hypertension (OR=2.98, 95% CI: 1.16–7.64) and lacunar infarction (OR=2.39, 95% CI: 1.19–4.81) were also associated with deep/mixed CMBs in Model 2 (Table 3).

Discussion

In our cross-sectional study, we found that the prevalence of CMBs in stroke-free community elderly was 12.6% and increased with age, similar to 10.6% in Shunyi study[6] and 15.3% in Rotterdam Scan Study.[17] To reduce confounding factors, the target populations in our study are stroke-free and non-demented elderly, representing a comparatively

natural aging situation. In this cohort, we found a higher prevalence of deep/mixed CMBs than strictly lobar CMBs, supporting the findings of more deep/mixed CMBs distribution in East than West.[3] And this feature prevents us from further analysis of risk factors in strictly lobar CMBs due to the limited sample.

The most interesting finding of our study was that we found two risk factors, APOE ε4 and CR1 non-F/F isoforms (F/S, S/S and F/F' isoforms) were associated with all CMBs and deep/mixed CMBs. APOE ε4, enhancing the deposition of amyloid and more frequently in patients with CAA,[18] has been widely reported the relationship with CMBs, usually strictly lobar CMBs.[7,8] CR1 has been found to increase the risk of late-onset Alzheimer's disease (AD) and CAA[15,18] and it was reasonable to hypothesize that the association of CR1 isoforms was probable more prominent with strictly lobar CMBs. But more than one study found inconsistent results on the

relationship between APOE/CR1 and CMBs. Caunca et al showed no statistical difference in CMB distribution across APOE genotype.[19] Li et al found that APOE ε4/ε4 genotype was a predictor for progression of cerebral microbleeds, regardless of location.[20] What is more, CR1 polymorphism increased cardiovascular risks,[21,22] which implies the relationship with deep/mixed CMBs.

We think that lipid metabolism and inflammation (systemic or vascular) might explain the association between APOE/CR1 and deep/mixed CMBs. Dyslipidemia is a known risk factor for cardiovascular diseases, including atherosclerosis (AS). AS may lead to CMBs, especially in deep region,[23] during the formation of collateral compensation in neovascularization. ApoE4, one lipid transporter preferring to binding large (30–80 nm), triglyceride-rich very low density lipoprotein (VLDL), is associated with elevated low density lipoprotein (LDL) levels and increases the risk for atherosclerosis.[24] Recent studies found that CR1 on erythrocytes may be involved in the clearance of atherogenic apolipoprotein B-containing lipoprotein, like LDL.[25]

In addition, inflammation has a close relationship with small vessel diseases, including CMBs.[26] It was shown that vascular inflammation was more associated with deep/mixed CMBs and systemic inflammation for lobar CMBs.[26] However, it is sometimes very difficult to ascertain whether both types of inflammation are interacted with each other. For example, long-term or severe systemic inflammation may also contribute to vascular inflammation or vice versa. APOE ε4 has been found to increase inflammation by accelerating endoplasmic reticulum stress and reducing macrophage function.[27] Also, APOE ε4 carriers were tended to produce a stronger neuroinflammatory responding to peripheral systemic inflammation.[28] CR1, a receptor for complement C3b/C4b, is also a central or peripheral factor involving complement mediated inflammation. Most (~80%) CR1 is expressed on erythrocyte, which implies the stronger immunoregulation function in peripheral circulation. CR1 is also expressed on vascular endothelial cells,[29] which may be involved in the induction and progression of atherosclerosis by influencing the assembly of the terminal complement C5b-9 complex.[30] This systemic or vascular inflammation or immunoregulation function of APOE/CR1 may explain the relationship with deep/mixed CMBs.

Cerebral small vessel disease has heterogeneous manifestations, including an ischemic phenotype (lacunar infarction, white matter hyperintensities) and a hemorrhagic one (microbleeds).[31] We found that white matter hyperintensities

increased the risk of all CMBs and deep/mixed CMBs, and lacunar infarction was only associated with deep/mixed CMBs. Consistently, Rotterdam Scan Study[17] found that both white matter lesions and lacunar infarcts increased the risk of CMBs, especially deep or infratentorial microbleeds. The close relationship between ischemic lesions and hemorrhagic tendency in brain parenchyma may be due to the interconnected networks formed by brain microvasculature.[31]

Diabetes may lead to microstructural changes and one study reported the association with multiple (≥2) CMBs.[32] However, we observed the protective effect for overall CMBs, which was hard to explain and need further study. One study focusing on the elderly communities found diabetes was the protective factor of strictly lobar CMBs (OR=0.69, 95% CI: 0.41–1.17), but no statistical significance (p= 0.17).[8] Another study found the protective effect of diabetes medication.[19]

Although the prevalence of CMBs increased with age in our study, we did not find the association of age with CMBs in Model 2, which might be due to small sample size or counteraction by white matter changes and lacunar infarctions. We confirmed the association between hypertension and deep/mixed CMBs, consistent with many other studies.[8] We also found the protective effect of antithrombotics (mainly aspirin) in Model 1 but did not remain significant in Model 2. Several studies showed that antithrombotics did no harm in individuals with less than five CMBs.[33] However, long-term use of aspirin or clopidogrel also increased the risk of CMBs.[34] It remained controversial for how much risk of CMBs contributed to therapy-induced cerebral hemorrhage. We did not observe the association of statin use with CMB presence as previously reported.[35]

There are several limitations of our study. Firstly, the sample size is small and only 199 people were included in our study. Due to the low prevalence of CMBs, the power of the test was limited and further analysis on strictly lobar CMBs was ceased due to poor goodness-of-fit. Secondly, a semiquantitative method was used to assess white matter hyperintensity on MRI scans. However, the inter-rater reliability between the countries for MRI evaluation was excellent.[12] Thirdly, some other risk factors of CMBs were not included, such as dyslipidemia and inflammation markers (eg, CRP).

Conclusion

Our study found that multiple factors including hypertension, CSVD (lacunar infarction and white matter hyperintensities), and genetic factors (APOE and CR1) increased

the risk of CMBs in a Chinese stroke-free non-demented community elderly. These findings might help to understand the mechanism underlying CMBs and prevent cerebrovascular disease in Chinese elderly.

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Shanghai Jiao Tong University School of Medicine ((2011) ethics committee No.13).

Consent to Participate

Written informed consent was obtained from each participant of this study.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no conflicts of interest to declare.

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