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ORIGINAL ARTICLE

Genome-Wide Association Study of Cerebral Microbleeds on MRI

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Abstract

Cerebral microbleeds are the presence of a group of pathological processes affecting the small arteries, arterioles, capillaries, and venules of the brain. Previous studies showed that cerebral microbleeds were associated with higher risk of dementia and stroke. We conducted a genome-wide association study of cerebral microbleeds to identify novel loci associated with the presence and progression of cerebral microbleeds. This study included 454 individuals composed by 176 subjects with cerebral microbleeds and 278 subjects without cerebral microbleeds in a non-Hispanic/Latino white population. Association of genetic variants with the presence and progression of cerebral microbleeds was assessed by logistic regression model. Potential genetic risk variants Apolipoprotein E (ApoE) polymorphisms were independently genotyped and checked the association with the presence and progression of cerebral microbleeds. No single-nucleotide polymorphisms (SNPs) associated with the presence or progression of cerebral microbleeds were identified at genome-wide significant level ($P < 1 \times 10^{-8}$). A total of 19 SNPs were associated with the presence of microbleeds at suggestive level ($P < 1 \times 10^{-5}$). One SNP was associated with lower progression risk for cerebral microbleeds with suggestive evidence ($P < 1 \times 10^{-5}$). ApoE ε4ε4 was independently associated with the presence and progression of cerebral microbleeds (odds ratio = 2.54, 95% confidence interval 1.08–6.00 and odds ratio = 5.1, 95% confidence interval 1.36–19.16). We highlighted 19 novel SNPs associated with the presence of cerebral microbleeds and one novel SNP associated with the progression of cerebral microbleeds for the first time. ApoE ε4ε4 was confirmed independently associated with the presence and progression of cerebral microbleeds.

Keywords ApoE · CMBs · Cerebral small vessel disease · GWAS · Genetic risk

Introduction

Cerebral microbleeds are 2–10-mm single small punctate areas of hypointensity with associated blooming detected on T2* weighted gradient-recalled echo* (GRE) or susceptibility-weighted magnetic resonance image (MRI) (Cordonnier et al. 2007; Wardlaw et al. 2013). Microbleeds can be perceived in normal aging population (Caunca et al. 2016), as well as patients with cerebrovascular disease (Shoamanesh et al. 2015) or dementia (Akoudad et al. 2016). A large cohort of community-dwelling aging people revealed that the overall prevalence of microbleeds was 15.4% in people aged 45 years or older and 35.7%in participants aged over 80 years old (Poels et al. 2010). Furthermore, cerebral microbleeds burden were associated with the increased risk of dementia (hazard ratio, 2.10; 95% CI, 1.21–3.64) (Akoudad et al. 2016) and all-cause stroke (hazard ratio, 1.93; 95% CI, 1.25–2.99) (Akoudad et al. 2015), indicating that cerebral microbleed was a crucial marker for neurodegenerative and cerebrovascular pathology. Thus, uncovering the fundamental mechanisms of microbleeds is of great importance for prevention, treatment, rehabilitation of age-related decline, cognitive deterioration, and stroke.

Identifying genetic risk factors can enhance our understanding of the mechanisms of how microbleeds develop and how microbleeds influence the functions of brain. The

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major vascular pathological changes of cerebral microbleeds include hypertensive vasculopathy and cerebral amyloid angiopathy (Greenberg et al. 2009), and both of them were related with complex genetic pathogenesis. Candidate gene studies demonstrated that Apolipoprotein E (ApoE) gene variant was associated with cerebral microbleeds (Poels et al. 2010; Schuur et al. 2011; Sveinbjornsdottir et al. 2008; Vernooij et al. 2008). ApoE gene has 3 common alleles (ε2, ε3, ε4) (Verghese et al. 2011), among which ε4 has significant association with cerebral microbleeds. Two meta-analyses demonstrated that ApoE ε4 carrier status was associated the presence of microbleeds, especially lobar microbleeds (Maxwell et al. 2011; Schilling et al. 2013). On the contrary, two independent cohort studies illustrated that no significant influence of ApoE genotypes in cerebral microbleeds was found in non-demented population (Lyall et al. 2015; Seifert et al. 2006). Furthermore, family studies identified several monogenic mutations were associated with cerebral small vascular diseases, presented by NOTCH3 in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Joutel et al. 1996). Other genes including HTRA1, COL4A1/A2, TREX1, and FOXC1/PITX2 were also found associated with cerebral microbleeds (Tan et al. 2017). However, these previously reported single genes were far from explaining the complex genetic mechanisms of cerebral microbleeds. Genome-wide association study (GWAS) is a powerful approach to identify the underlying genetic risk factors of this kind of complex trait. To the best of our knowledge, there was no genome-wide association study elaborating the underlying genetic basis of cerebral microbleeds.

Here we conducted the first genome-wide association study of cerebral microbleeds to find out the genetic risk factors, aiming at identifying variants associated with the presence and progression of cerebral microbleeds and providing implications for further exploring the pathophysiology of cerebral microbleeds.

Methods

Study Design

We designed two whole genome-wide association studies in the pattern of retrospective case-control study to discover common variants involved in the presence and progression of cerebral microbleeds. Firstly, we conducted a genome-wide association scan between the groups with microbleed presence and absence, seeking for the loci associated the appearance of microbleeds. Then, we screened out the patients with cerebral microbleeds who had at least two MRI scans during follow-up. Once the patients' cerebral microbleed count increased compared with baseline during the follow-up period, we define these patients as cerebral microbleed progression group. If the patients' cerebral microbleeds burden showed no progression, they would be categorized as non-progression group. The average follow-up time is shown in Table 1. GWAS was conducted between microbleed progression group and non-progression group to identify which loci were associated with the increase of cerebral microbleed count. The technical roadmap of the study is shown in Fig. 1.

Study Population

We screened out 530 non-Hispanic/Latino white participants with both microbleed count analysis and genome-wide SNP information from Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort (www.adni.loni.usc.edu). To avoid potential population stratification which will bring about confounding bias, we restricted all subject to non-Hispanic/Latino white people. We clearly defined the trait of case and control to maximize the opportunity of identifying the risk variants. Participants in case group exhibited definite cerebral microbleeds confirmed by strict neuroimage protocol which will be elaborated thoroughly in the following context. Participants in control group had neither

Table 1 Demographic and clinical characteristics of participants

	CMB absent ($n = 278$)	CMB present ($n = 176$)	P	CMB progression absent ($n = 95$)	CMB progression present ($n = 57$)	P
Age (mean ± SD)	72.7 ± 7.9	76.6 ± 7.3	< 0.05	75.0 ± 7.2	77.3 ± 6.3	< 0.05
Sex (male/female)	141/137	110/66	< 0.05	58/37	38/19	0.5
History of HTN (yes/no)	124/154	79/97	0.95	44/51	25/32	0.77
History of endocrine-metabolic disease (yes/no)	111/167	70/106	0.97	39/56	19/38	0.34
Smoking (yes/no)	120/158	64/112	0.15	36/59	17/40	0.31
Follow-up time (years)	–	–	–	3.38 ± 2.02	3.32 ± 1.90	0.84
Cognitive status (CN/MCI)	102/176	59/117	0.49	31/64	16/41	0.56

All subjects were restricted to not Hispanic/Latino, white

CMB cerebral microbleed, *CN* cognitive normal, *MCI* mild cognitive impairment, *SD* standard deviation

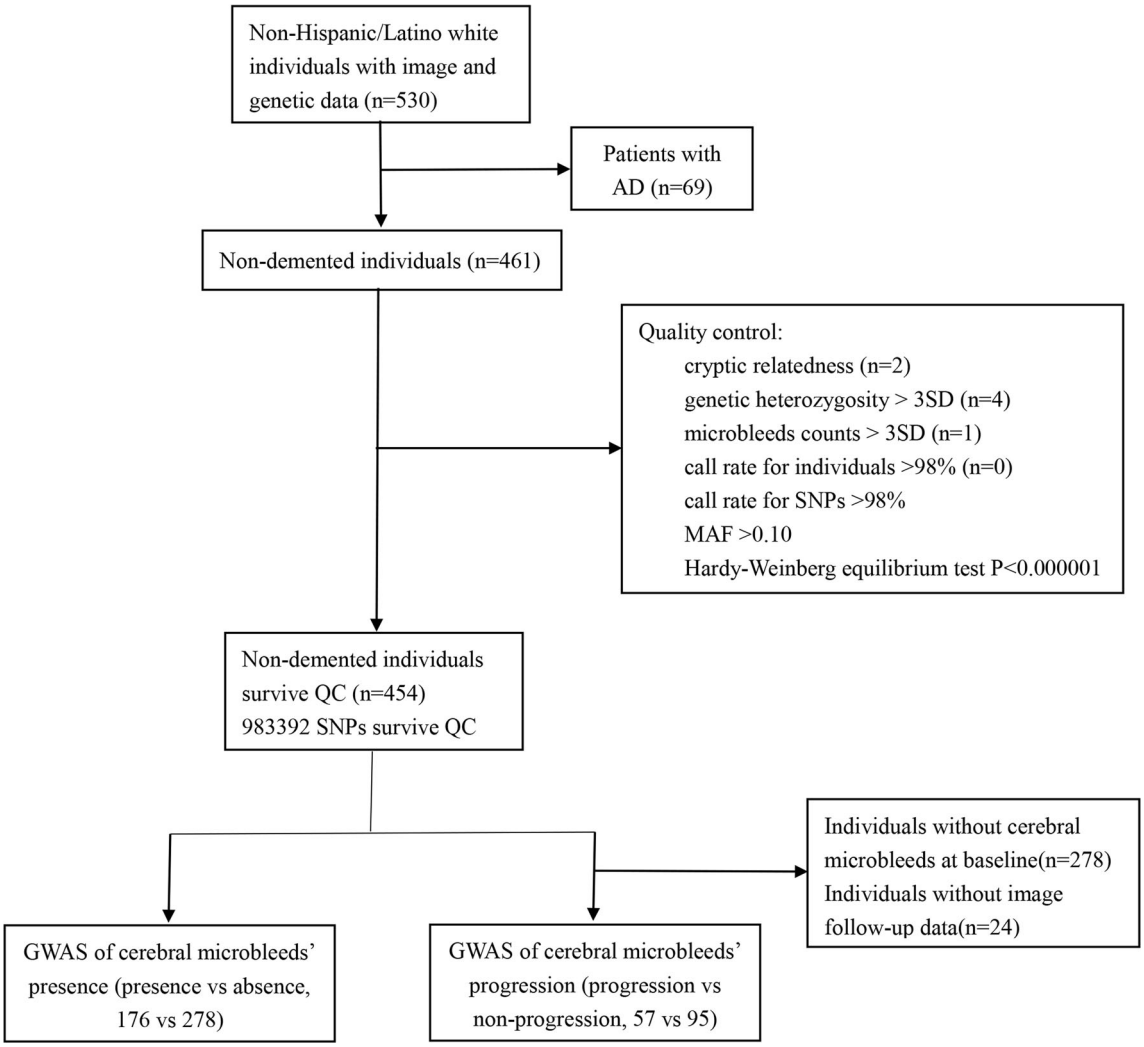


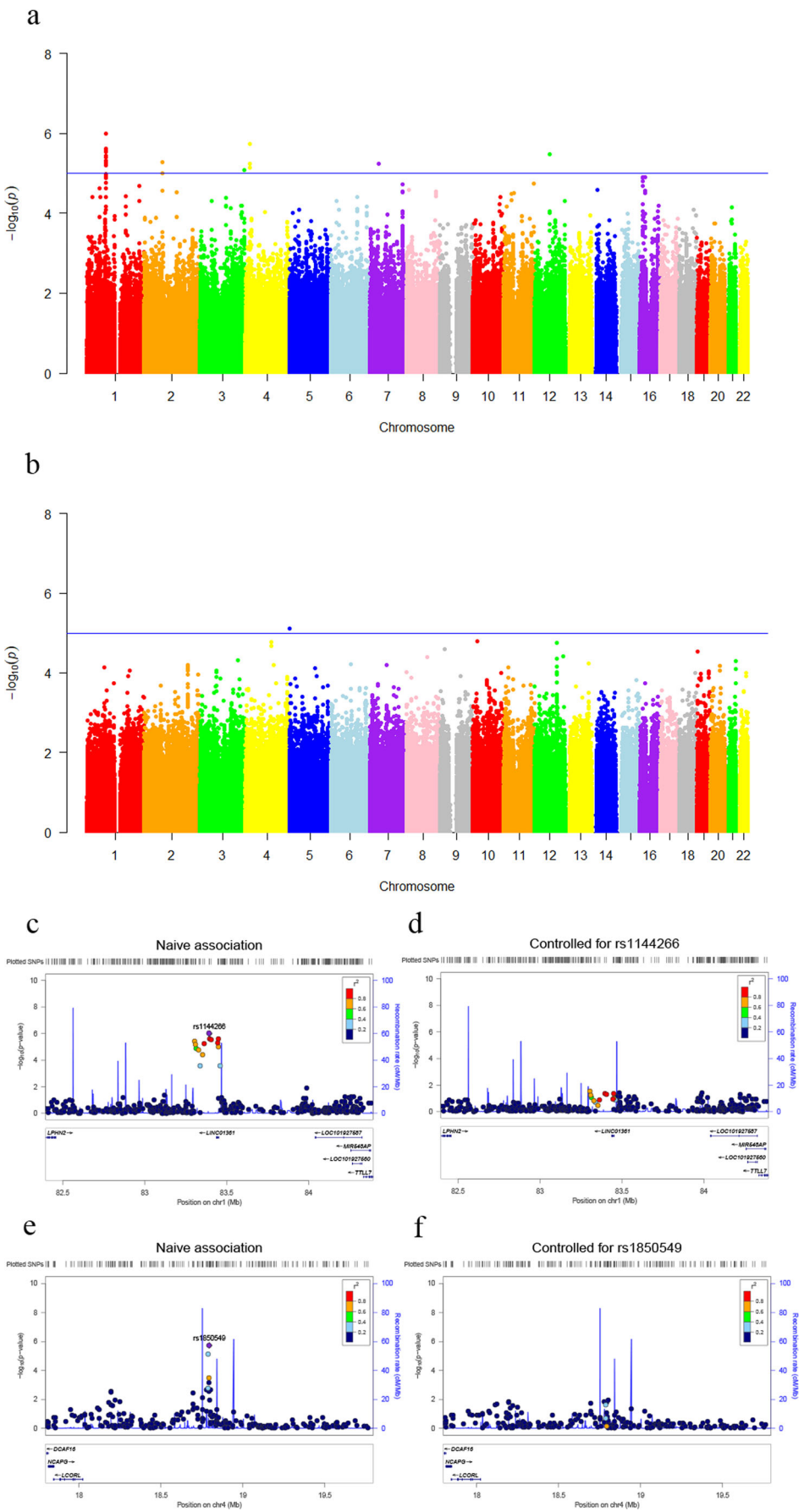
Fig. 1 Flow diagram for the study. AD Alzheimer disease, GWAS genome-wide association study, MAF minor allele frequency, QC quality control, SD standard deviation

cerebral microbleeds nor other pathological presence in MRI. Participants ($n = 69$) diagnosed as AD at baseline or at any point of follow-up period were excluded. Cryptic relatedness and genetic heterozygosity were checked by genomic identity-by-descent using PLINK (version 1.90 beta) (Purcell et al. 2007). Six participants were excluded owning to potential relationship ($n = 2$) or genetic heterozygosity > 3 standard deviation (SD, $n = 4$). We also excluded one patient with cerebral microbleed count > 3 SD, resulting in 454 valid samples.

Finally, we included 454 non-demented non-Hispanic/Latino white individuals (176 subjects with cerebral microbleed presence and 278 subjects with cerebral microbleed absence). All of them met data quality control criteria. One hundred and fifty-two participants with microbleed count follow-up data were included in the GWAS of cerebral microbleeds' progression. Among them, 57 patients had increased counts of cerebral microbleeds within the period of follow-up and 95 patients' microbleed count showed no progression. Detailed demographic information is demonstrated in Table 1.

ADNI Dataset

ADNI was a multisite longitudinal study including normal control individuals, patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD) in America and Canada. This dataset aimed at exploring the mechanisms for AD, detecting early signs of AD, and validating biomarkers for AD (Toga and Crawford 2015). ADNI dataset collected participants' clinical data, neuroimaging data, genetic data, and biospecimens. More than 1500 individuals were enrolled in this project which offered researchers mounting clinical information and long-term follow-up data.


a
b
Chromosome
c
Naive association
d
Controlled for rs1144266
e
Naive association
f
Controlled for rs1850549

◀ **Fig. 2** Genome-wide association results of the cerebral microbleed presence and progression. The Manhattan plot depicts $-\log_{10}$-transformed two-tailed P value of each SNPs on the Y axis and their genomic position on X axis. The horizontal blue line indicates suggestive threshold ($P = 1 \times 10^{-5}$). **a** SNP association results for the presence of cerebral microbleeds. **b** SNP association results for the progression of cerebral microbleeds. **c** Regional association results for 82.5 to 84 Mb region of chromosome 1. **d** Association results for 82.5 to 84 Mb region of chromosome 1 controlling for rs1144266. **e** Regional association results for 18 to 19.5 Mb region of chromosome 4. **f** Association results for 18 Mb to 19.5 Mb region of chromosome 4 controlling for rs1850549

ADNI study obtained ethics approval from institutional review boards of all participating institutions, and written informed consent from participants or authorized representatives.

Neuroimaging Analysis

A standardized 3T MRI protocol including T2* GRE and T1-weighted 3D MPRAGE sequences was applied to each subjects (http://adni.loni.usc.edu/methods/documents/mri-protocols/). The GRE sequence parameters were as the following: TE, 20 ms; TR, 650 ms; flip angle, 20°; section thickness, 4 mm; and section gap, 0 mm. In MRI, cerebral microbleeds were defined as homogenous hypointense lesions up to 10 mm in diameter in the white or gray matter on T2* GRE images as described previously (Kantarci et al. 2013). Images were graded manually facilitated by software in a single quality-control center (Gunter et al. 2009). Manual raters assessed all available T2* images to detect the presence of cerebral microbleeds. To control the image analysis quality, the anomalies would be double checked by trained analysts or radiologists. A list of summary including image finding, lesion location, and basic clinical information was reported. Three values (possible, definite, rescinded) were used to describe the status of the finding. In this study, we only included individuals with definite cerebral microbleeds as cases and individuals without cerebral microbleeds or other pathological findings as controls.

Table 3 The distribution of ApoE genotypes in patients with or without cerebral microbleed presence and progression

	ApoE genotype					
	ε2ε2	ε2ε3	ε2ε4	ε3ε3	ε3ε4	ε4ε4
CMB absent	0	24	4	160	78	12
CMB present	0	18	3	87	54	14
CMB progression absent	0	13	2	47	29	4
CMB progression present	0	5	0	27	16	9

APOE apolipoprotein E, *CMB* cerebral microbleed

Genotyping and Quality Control

Each individual was genotyped by Illumina Omni2.5M BeadChip in accordance with manufacturer's protocols. We performed standard stringent quality control with PLINK software. The quality control criteria for GWAS of microbleed presence and progression were both as the following: call rate for SNPs > 98%, call rate for individuals > 98%, minor allele frequency (MAF) > 0.10, and Hardy-Weinberg equilibrium test $P < 0.000001$. Due to relatively small sample size, we restrict SNPs' MAF > 0.1 to decrease false-positive likelihood. Finally, 983,392 genotyped variants were included in cleaned dataset of microbleed presence study, and those in microbleed progression study were 979,539 variants. The Illumina BeadChip did not contain SNPs rs429358 and rs7412 which define the ApoE alleles, and therefore, the polymorphisms of ApoE were genotyped by kit separately.

Statistical Analyses

Group difference in demographic and clinical characteristics was evaluated by Pearson chi-squared test for categorical variables and independent-samples T test for continuous variable. Age, sex, history of endocrine-metabolic syndrome, history of hypertension, history of smoking, and follow-up period were included as covariates if $P < 0.05$ between two groups in the regression model. Principal components were calculated by GCTA (version 1.91.6beta) as covariant (Yang et al. 2011). Then, we used PLINK to perform association study between cerebral microbleed presence and genetic variants with logistic regression model adjusted by microbleeds' risk factor and principal components. Furthermore, we used a logistic regression model to elaborate the association between cerebral microbleed progression and genetic variants adjusted by microbleeds' risk factor and principal components. P value = 5×10^{-8} was considered as significant threshold and P value = 1×10^{-5} was set as the suggestive threshold, adjusting multiple test by Bonferroni correction, which were in accordance with generally acknowledged GWAS standard (Hayes 2013). We used R package qqman to visualize genome-wide association results (R, version 3.4.1; The R Foundation). Using LocusZoom web tool (Pruim et al. 2010), regional associations were visualized. All statistical analyses mentioned above were performed by SPSS (version 25, IBM) and PLINK.

Results

Demographic and Clinical Characteristics

The demographic and clinical characteristics of all included subjects are illustrated in Table 1. In brief, 176 patients (110

Table 4 Association between ApoE ε2 and ε4 and cerebral microbleed presence and progression

	CMB presence		CMB progression	
	OR (95% CI)	Yes/no	OR (95% CI)	Yes/no
ApoE ε2 allele				
0	1.0 (Reference)	155/250	1.0 (Reference)	52/80
1	1.36 (0.72–2.53)	21/28	1.84 (0.62–5.51)	5/15
ApoE ε4 allele				
0	1.0 (Reference)	105/184	1.0 (Reference)	32/60
1	1.42 (0.92–2.19)	57/82	1.07 (0.49–2.30)	16/31
2	2.54 (1.08–6.00)*	14/12	5.1 (1.36–19.16)*	9/4

ApoE apolipoprotein E, *CMB* cerebral microbleed, *OR* odds ratio
*$P < 0.05$

men, 76.6 ± 7.3 years) with cerebral microbleeds and 278 patients (141 men, 72.7 ± 7.9 years) without cerebral microbleeds or other pathological neuroimage findings were enrolled in GWAS of microbleed presence. There was no statistical difference in baseline characteristics except for age and sex between two groups. Fifty-seven patients (38 men, 77.3 ± 6.3 years) were detected cerebral microbleed count increased, while 95 patients (58 men, 75.0 ± 7.2 years) showed no progression of microbleeds. Similarly, the baseline characteristics were matched between two groups except for age. The average follow-up time also showed no significant difference between two progression group and non-progression group (progression presence vs progression absence, 3.38 ± 2.02 vs 3.32 ± 1.90 years, $P = 0.84$).

SNPs Associated with the Presence and Progression of Cerebral Microbleeds

A total of 454 non-demented non-Hispanic/Latino individuals were identified for GWAS of cerebral microbleed presence. The genomic inflation factor was 1.00, demonstrating no remarkable inflation caused by population stratification. Manhattan plot (Fig. 2a) showed the overall genome-wide association results. After adjusting for age, sex and the first three principal components, 19 SNPs on six chromosomes (see Table 2) were identified to be associated with the presence of microbleeds at suggestive levels of $P < 1 \times 10^{-5}$, while no SNPs reached genome-wide significance level. Fifteen SNPs were located in the intron of known genes, and 4 SNPs lied in non-coding area outside of known gene. Since 11 SNPs were located in chromosome 1 nearly, and four SNPs lied in chromosome 4 were not far away. We analyzed SNPs mapped closely to the tops two suggestive SNP (rs1144266, rs1850549) region. Regional analysis illustrated that these SNPs associated with cerebral microbleed presence were no longer significant after controlling the two suggestive SNPs (Fig. 2c, d and e, f), indicating that associations in this region were driven by rs1144266 and rs1850549. The minor allele of rs1144266 (C) in the intron of LINC01362, the minor allele of rs12497385 (T) in the intron of LOC105374287, and the minor allele of rs10263645 (C) in the intron of AMPH were associated with lower onset risk of the presence of cerebral microbleeds (OR = 0.45, 0.51, and 0.41 separately). The minor allele of rs1368908 (A) in the intron of CTNNA2, the minor allele of rs1850549 (T) in the intron of LOC105374510, and the minor allele of rs6581525 (G) in the intron of LINC01362 showed the association of onset risk of microbleeds (OR = 2.21, 2.56, and 2.05 separately).

We further analyzed the SNPs associated with the progression of cerebral microbleeds. The minor allele of rs55738218 (G) was associated with lower progression risk at suggestive level (OR = 0.22). Manhattan plot (Fig. 2b) depicted that only one SNP reaches the suggestive level, and no SNPs showed association with the progression of cerebral microbleeds at genome-wide significant level. The genomic inflation factor is 1.04, indicating no significant evidence of population stratification.

ApoE ε4ε4 Is Associated with the Presence and Progression of Cerebral Microbleeds

ApoE was considered to be a potential risk factor for cerebral microbleeds previously but without validating evidence. We validated the association between ApoE alleles and cerebral microbleeds. The distribution of ApoE genotypes in patients with or without cerebral microbleed presence and progression is demonstrated in Table 3. After adjustment for age, sex, history of hypertension, history of endocrine-metabolic disease, and history of smoking, ApoE ε4ε4 was independently associated with the presence of cerebral microbleeds (OR = 2.54, 95% CI 1.08–6.00) (Table 4). Carrying one ε4 allele would not increase the risk of microbleeds. Meanwhile, ApoE ε4ε4 was also independently associated with the progression of cerebral microbleeds (OR = 5.1, 95%CI 1.36–19.16). On the contrary, ApoE ε2 allele showed no association with either presence or progression of cerebral microbleeds in analyses.

Discussion

We performed the first genome-wide association study of cerebral microbleeds in non-demented cohort, which highlights 19 SNPs associated with the presence of cerebral microbleeds and one SNP associated with the progression of cerebral microbleeds at suggestive level ($P < 1 \times 10^{-5}$). We also validated that ApoE ε4ε4 was an independent genetic risk factor for the presence and progression of cerebral microbleeds.

Table 2 Top SNPs associated with cerebral microbleeds' presence (P values < 1×10^{-5})

CHR	SNP	Minor allele	MAF	Closest gene	Gene functions	SNP location	OR	P
1	rs1144266	C	0.34	LINC01362	Unknown	Intron	0.45	1.01×10^{-6}
4	rs1850549	T	0.17	LOC105374510	Unknown	Intron	2.56	1.84×10^{-6}
1	rs10493734	T	0.33	LINC01361	Unknown	Intron	0.46	2.43×10^{-6}
1	rs1144267	T	0.34	LINC01362	Unknown	Intron	0.47	2.49×10^{-6}
1	rs7411897	A	0.34	LINC01362	Unknown	Intron	0.47	2.83×10^{-6}
12	rs6581525	G	0.38	SRGAP1	Regulating neuronal migration	Intron	2.05	3.39×10^{-6}
1	rs11163625	G	0.34	LINC01362	Unknown	Intron	0.47	3.69×10^{-6}
1	rs12132310	A	0.28	LOC107985396	Unknown	438 k	0.46	3.92×10^{-6}
1	rs10782802	G	0.34	LINC01361	Unknown	Intron	0.48	4.85×10^{-6}
2	rs1368908	A	0.24	CTNNA2	Loss of CTNNA2 lead to defect in neurite stability and migration	Intron	2.21	5.18×10^{-6}
1	rs1348045	A	0.34	LINC01362	Unknown	Intron	0.48	5.38×10^{-6}
7	rs10263645	C	0.21	AMPH	Encoding amphiphysin which is required for dendrite maturation	Intron	0.41	5.65×10^{-6}
1	rs11163602	G	0.34	LINC01362	Unknown	461 k	0.48	5.66×10^{-6}
4	rs66690887	A	0.28	LOC105374510	Unknown	Intron	2.05	5.85×10^{-6}
4	rs67159217	T	0.28	LOC105374510	Unknown	Intron	2.05	5.85×10^{-6}
1	rs12140057	A	0.28	LOC107985396	Unknown	456 k	0.47	6.31×10^{-6}
1	rs11163585	T	0.28	LOC107985396	Unknown	443 k	0.47	6.31×10^{-6}
4	rs10027565	A	0.39	LOC105374510	Unknown	Intron	1.97	7.32×10^{-6}
3	rs12497385	T	0.47	LOC105374287	Unknown	Intron	0.51	8.46×10^{-6}

CHR chromosome, *MAF* minor allele frequency, *SNP* single nucleotide polymorphism

Among 19 SNPs identified associated with cerebral microbleeds, the function of SNPs within or near LINC01362, LINC01361, LOC107985396, LOC105374287, LOC105374510 was not reported in previous literatures. The variation rs6581525 was located in the intron of SRGAP1 which coded SLIT-ROBO Rho GTPase–activating protein 1, regulating the development of nervous system (Bacon et al. 2009; Ip et al. 2011). It has been reported that SRGAP1 was involved in the process of focal cerebral infarction and neuroinflammation (Dai et al. 2015; Sherchan et al. 2016). Rs1368908 was located within the intron of CTNNA2 which encoded αN-catenin, and it was associated with the onset of cerebral microbleeds. Biallelic loss of CTNNA2 could lead to defects in neurite stability and migration (Schaffer et al. 2018). αN-catenin coded by CTNNA2 was an important protector against cerebral infarction, and it played a critical role in recovery by supporting the neurovascular unit and integrity of brain parenchyma (Posada-Duque et al. 2014). AMPH containing rs10263645 encodes amphiphysin, a protein associated with the cytoplasmic surface of synaptic vesicles, which was a required component for dendrite maturation (Sheng et al. 2018). Furthermore, autoantibody against amphiphysin was found in subjects with neuropsychiatrical disease including stroke (Dahm et al. 2014). In conclusion, SRGAP1, CTNNA2, and AMPH play significant roles in the function of central nervous system, which imply that the identified genes loci were essential for understanding the pathogenesis of cerebral microbleeds. We also discovered rs55738218 within SLC12A7 was associated with the progression of cerebral microbleeds, which may be a genetic predictor for microbleeds burden progression. However, due to the small sample size of GWAS for progression of microbleeds, we need further study to confirm that.

Our results showed that ApoE ε4ε4 not only is associated with the onset of cerebral microbleeds but also is a predictor for progression of cerebral microbleeds for the first time, regardless of location. Previous studies showed that APOE ε4 carrier was associated with higher prevalence of cerebral microbleeds, especially strict lobar microbleeds (Loehrer et al. 2014; Maxwell et al. 2011; Romero et al. 2014; Schilling et al. 2013). On the contrary, several studies illustrated that no statistical association between ApoE genotype and the distribution and presence of cerebral microbleeds was found (Caunca et al. 2016; Jeerakathil et al. 2004; Lyall et al. 2015; Rabelo et al. 2017). However, these studies did not mention the association between double ApoE ε4 alleles with cerebral microbleeds, no matter where they located. Our results also showed that one ApoE ε4 allele was not associated with neither the presence nor progression of cerebral microbleeds which consisted with the aforementioned research. And we found that double ε4 carriers were associated

with higher risk of the presence and progression of cerebral microbleeds. As for the underlying mechanism, ApoE ε4 allele carrier was associated with cerebral amyloid angiopathy (CAA) which was characterized by Aβ accumulation in cortical arterioles leading to vessel lumen reduction and microbleeds (Makela et al. 2016). The association between ApoE ε4 and CAA supported that ApoE ε4 was a risk factor of lobar microbleeds. Furthermore, Rotterdam scan study found that the patients with hypertension and ApoE ε4 allele had the highest risk of white matter lesion (de Leeuw et al. 2004). White matter lesion is also an important image biomarker of cerebral small vessel disease, and it shares some fundamental pathophysiological mechanism with cerebral microbleeds. Thus, we can imply that ApoE ε4 may be a risk factor of hypertensive angiopathy.

Our study has several strengths. This is the first GWAS study focused on discovering novel genetic variants, influencing the presence and progression of cerebral microbleeds in a non-demented sample from multiple medical centers. High-quality standardized MRI protocols were uniformly employed in all research centers, and the final image analyses were performed in one lab which maximized the accuracy of determination of cerebral microbleeds. The present study also has several limitations. The modest sample size limited the efficacy of finding mini effect variants. A larger sample size study is proposed to detect the potential genetic risk variants. Furthermore, although the average follow-up time showed no difference between two groups, the follow-up periods varied from individuals. The subjects classified into non-progression group may be due to short follow-up periods. Thus, longer and unanimous follow-up time is needed to find out the risk factors for the progression of cerebral microbleeds.

Conclusion

No SNPs were identified to be associated with cerebral microbleeds at genome-wide significant level, indicating the genetic heterogeneity of cerebral small vessel disease. We detected 19 novel SNPs that are associated with cerebral microbleed presence and one SNP that is associated with cerebral microbleed progression at the suggestive level. Furthermore, we figured out that ApoE ε4ε4 was independently associated with both presence and progression of cerebral microbleeds. Further works to uncover the causal relationship between these identified polymorphisms are highly warranted based on our results' implications.

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Compliance with Ethical Standards

ADNI study obtained ethics approval from institutional review boards of all participating institutions, and written informed consent from participants or authorized representatives.

Competing Interests The authors declare that they have no conflict of interest.

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