ALDH2 rs671 polymorphisms and the risk of cerebral microbleeds in Chinese elderly: the Taizhou Imaging Study

Zhen Zhu\(^1,2,\#\), Yanfeng Jiang\(^2,3,\#\), Mei Cui\(^4\), Yingzhe Wang\(^4\), Shuyuan Li\(^5\), Kelin Xu\(^2,6\), Kexun Zhang\(^1,2\), Chengkai Zhu\(^3,7\), Wanghong Xu\(^1\), Li Jin\(^2,3,7\), Weimin Ye\(^2,8\), Chen Suo\(^1,2\), Xingdong Chen\(^2,3\)

\(^1\)Department of Epidemiology, School of Public Health, and the Key Laboratory of Public Health Safety of Ministry of Education, Fudan University, Shanghai 200438, China; \(^2\)State Key Laboratory of Genetic Engineering, Human Phenome Institute, Fudan University, Shanghai 200438, China; \(^3\)Fudan University Taizhou Institute of Health Sciences, Taizhou 225312, China; \(^4\)Department of Neurology, Huashan Hospital, Fudan University, Shanghai 200030, China; \(^5\)International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200030, China; \(^6\)School of Data Science and Institute for Big Data, and the Collaborative Innovation Center for Genetics and Development, Fudan University, Shanghai 200433, China; \(^7\)School of Life Sciences, Fudan University, Shanghai 200438, China; \(^8\)Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden

Contributions: (I) Conception and design: X Chen, C Suo, Y Jiang; (II) Administrative support: X Chen, C Suo, Y Jiang; (III) Provision of study materials or patients: L Jin, X Chen; (IV) Collection and assembly of data: Z Zhu, Y Jiang, K Xu, K Zhang; (V) Data analysis and interpretation: Z Zhu, Y Jiang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

\#These authors contributed equally to this work.

Correspondence to: Xingdong Chen, PhD. School of Life Sciences, Fudan University, Songhu Road 2005, Shanghai 200438, China.
Email: xingdongchen@fudan.edu.cn; Chen Suo, PhD. School of Public Health, Fudan University, Dong’an Road 138, Shanghai 200032, China.
Email: suochen@fudan.edu.cn.

Background: Cerebral microbleeds (CMBs) are more prevalent in Asian populations, and have been associated with increased risk of stroke, dementia and mortality. So far, risk factors for CMBs other than hypertension were merely known. Previous studies have shown that polymorphisms at aldehyde dehydrogenase 2 (ALDH2) gene were independently associated with the risk of stroke. Its role in CMBs, however, remains unclear. This study aimed to evaluate the associations of ALDH2 gene polymorphisms with CMBs in Chinese elderly.

Methods: Using bio-specimen and data collected at baseline survey of the population-based Taizhou Imaging Study (TIS) (phase I), we genotyped the single nucleotide polymorphisms (SNPs) at ALDH2 among 549 individuals aged 55–65 years, and rs671 was used as surrogate marker of ALDH2. CMBs were detected on brain magnetic resonance imaging (MRI), and further categorized as strictly lobar or as deep/mixed. Logistic regression models were used to evaluate the associations of the variants at ALDH2 and CMBs.

Results: CMBs were present in 103 individuals (18.8%). Forty-one point three percent participants were with ALDH2 *2 allele and 5.1% had ALDH2 *2/*2 genotype. Subjects with ALDH2 *1 allele were more likely to be drinker, have hypertension or CMBs than those with *2 allele (all P<0.05). Multivariate logistic regression model showed that the ALDH2 *1/*1 genotype was independently associated with CMBs (P=0.013), particularly for deep/mixed CMBs (P=0.008), and the association was more pronounced in men, non-drinkers or hypertension patients.

Conclusions: The results suggest that Han Chinese with ALDH2 *1/*1 genotype may be more susceptible to CMBs than those with ALDH2 *2 allele.

Keywords: Cerebral microbleeds (CMBs); aldehyde dehydrogenase 2 (ALDH2); genetic association study; brain magnetic resonance imaging (brain MRI)

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Introduction

Stroke accounts for approximately 10% of all-cause deaths globally, and the estimated lifetime risk of stroke was about 25% after 25 years old (1,2). Eastern Asia ranks first in incidence of stroke, particularly for China where stroke is the leading cause of death (2,3). Fortunately, there exists a long subclinical phase of stroke that can be detected by neuroimaging, making it possible for intervention and prevention of the fatal disease.

Recently, cerebral small vessel disease (CSVD) has been identified as a stroke-related preclinical vascular disease in the brain (4,5). Cerebral microbleeds (CMBs), a magnetic resonance imaging (MRI) marker of CSVD, have been found quite common in patients with stroke, as well as in general elderly population (5). Contrast to other CSVD markers such as lacune, white matter hyperintensity, and perivascular space, CMBs were observed to indicate both ischemic and hemorrhagic changes in the brain (4). Multiple epidemiological studies have demonstrated that CMBs are associated with increased risk of stroke (6), dementia (7) and mortality (8). So far, however, little is known about risk factors other than hypertension for CMBs, especially in general populations.

Considering the close association between heavy alcohol drinking and the risk of stroke (9), which can be modified by aldehyde dehydrogenase 2 (ALDH2), a so-called alcohol flush gene (10), several studies have focused on the influence of variants at ALDH2 on cerebrovascular disease (11-17). However, the results were inconsistent (11-17). ALDH2 gene encodes the major enzyme in ethanol oxidation process (18), and the allelic variant *2 at the gene was observed to dramatically reduce enzyme activity. The ALDH2 *2 allele is more prevalent in Asians than that in Caucasians (19), leading to less alcoholism in Asians (20) but the highest incidence of stroke. The unparallelism suggests that Asian may be more susceptible to stroke, particularly for those with ALDH2 *1 allele.

Herein, based on the population-based Taizhou Imaging Study (TIS), we conducted a cross-sectional study to evaluate the association between ALDH2 gene polymorphisms and CMBs, the MRI marker for CSVD, the stroke-related preclinical vascular disease in general population in China. Our study may provide evidence for prevention of stroke in the high-risk population.

Methods

Study design and participants

The present study was conducted as a part of the TIS, an ongoing community population-based neuroimaging cohort, which originates from the Taizhou Longitudinal Study (21). The detailed description of TIS has been reported in our previous studies (22,23). In brief, from March 2013 to January 2015, a total of 624 Han Chinese individuals aged 55–65 years without stroke, cancer, cardiovascular disease, psychiatric disorders, or other serious illness in two villages of Taixing were invited to participate the baseline survey of TIS, and received extensive physical, cognitive, and brain MRI examinations. Totally, 562 participants met the inclusion criteria were included in phase I of the TIS. Of these, 13 individuals without baseline whole blood samples were excluded and eventually 549 subjects were involved the present investigation. All related examinations were performed on the same day as the MRI examination at the Taizhou People’s Hospital. The TIS was approved by the Ethics Committee of the School of Life Sciences, Fudan University, Shanghai, China (institutional review board approval number: 469), and all participants gave written informed consent.

Brain MRI and CMBs assessment

All the participants underwent brain MRI on the same 3.0T scanner (Magnetom Verio Tim scanner; Siemens, Erlangen, Germany) followed a pre-determined protocol. Two experienced neurologists (M.C. and Q.Y.) independently diagnosed CMBs according to the Standards for Reporting Vascular Changes on Neuroimaging (STRIVE) (24) through Horos software (version 1.1.7). In general, CMBs were defined as round or ovoid shaped local signal loss on T2*-weighted GRE sequence and with 2−5 mm diameter (no more than 10 mm). The final CMBs diagnoses were based on their consensus and the final decision regarding to discrepancies were made by a senior neuroradiologist (W.J.T.). The kappa value of CMBs identification was 0.83. CMBs were divided into strictly lobar CMBs and deep/mixed CMBs by lesion location, as mixed CMBs shared similar characteristics with deep CMBs (25). The two typically CMBs location have their own pathologies distribution: strictly lobar CMBs usually located in frontal,
parietal, temporal, and occipital, whereas deep/mixed CMBs usually located in basal ganglia and thalamus, corpus callosum, and infratentorial including brain stem and cerebellum (25).

DNA collection and genotyping
Venous blood samples were drawn between 7:00 and 8:00 am after an overnight fasting by certified nurses in vacuum tubes with ethylenediaminetetraacetic acid (EDTA). DNA was extracted by using a whole-blood DNA extraction and purification kit (BaiO, Shanghai, China) according to the manufacturer's instructions. Genotyping was performed on coded genomic DNA samples using Axiom Precision Medicine Research Array (PMRA). All single nucleotide polymorphisms (SNPs) at ALDH2 and the previous reported surrogate marker of ALDH2 were extracted for preliminary analysis (Table S1). Results show that only rs2074356 and rs671 were associated with CMBs, however, rs2074356 is in high linkage disequilibrium with rs671 in our population (R^2=0.653). Therefore, the most well-known functional SNP rs671 (26), was selected as a surrogate ALDH2 genotyping marker for subsequent analyses. Since only a small number of individuals carry ALDH2 *2/*2 genotype (n=28, 5.1%) in this study, we combined ALDH2 *2/*2 and *1/*2 genotype as the reduced enzyme activity group, whereas participants with ALDH2 *1/*1 genotype as the fully enzyme activity group (15). The SNP rs671 had a 100% call rate in all samples, and were in consistency with Hardy-Weinberg Equilibrium among CMB-free participants (P=0.374).

Covariates
In-person interview was performed to collect demographic characteristics and risk factors using a structured interviewer-administered questionnaire which included age, sex, habits of smoking and alcohol drinking. Alcohol drinking was defined as alcohol use at least once per week within recent 6 months. Calculation of body mass index (BMI) and definitions of hypertension, diabetes mellitus (DM), and hyperlipidemia were described in our previous study (22).

Statistical analysis
The categorical variables were compared using Pearson's chi-squared test and were presented as frequencies (%). Continuous variables were described as mean (standard deviation, SD) and were compared using Student's t-tests. Univariate and multivariate logistic regression models were used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of CMBs with potential risk factors. Then we tested the multiplication interactions of ALDH2 genotypes with sex, alcohol drinking and hypertension status in the risk of CMBs. Stratification analyses were then conducted by sex, alcohol drinking, and hypertension status, using *2 allele as the reference group. In multivariate models, model 1 was adjusted for age, sex, BMI, smoking and alcohol drinking while model 2 was additionally adjusted for hypertension, DM, and hyperlipidemia. All analyses were performed using the R program (version 3.5.1, R core team), and two-tailed P<0.05 was considered as statistical significant.

Results
The baseline characteristics for CMBs locations (absence, deep/mixed or lobar CMBs) of the 549 participants are presented in Table 1. In total, 103 individuals (18.8%) were identified with CMBs, of whom 48 (46.6%) were

| Characteristics | Non-CMBs (n=446) | CMBS location |
|-----------------|-----------------|---------------|
| Age, mean (SD), years | 59.02 (2.72) | 59.71 (2.57) | 60.12 (2.56)* |
| Women, n (%) | 233 (52.2) | 33 (60.0) | 33 (68.8) |
| BMI, mean (SD) | 24.13 (3.32) | 24.45 (3.96) | 23.45 (2.16) |
| Smoking*, n (%) | 162 (36.7) | 18 (33.3) | 11 (22.9) |
| Alcohol drinking*, n (%) | 133 (30.3) | 15 (27.8) | 11 (22.9) |
| Hypertension, n (%) | 235 (52.7) | 38 (69.1)* | 32 (66.7) |
| Diabetes, n (%) | 61 (13.7) | 9 (16.4) | 3 (6.3) |
| Hyperlipidemia, n (%) | 216 (48.4) | 27 (49.1) | 21 (43.8) |
| ALDH2 genotype (rs671)*, n (%) | | | |
| *1/*1 | 249 (55.8) | 42 (76.4) | 31 (64.6) |
| *1/*2 | 173 (38.8) | 10 (18.2) | 16 (33.3) |
| *2/*2 | 24 (5.4) | 3 (5.5) | 1 (2.1) |

*5 subjects had missing data for smoking and drinking status; *, P<0.05 (compared with non-CMBs group). CMBs, cerebral microbleeds; SD, standard deviation; BMI, body mass index; ALDH2, aldehyde dehydrogenase 2.
lobar CMBs and 55 (53.4%) were deep/mixed CMBs. The participants with CMBs was slightly older than those without, with mean age of 59.90 vs. 59.02 years (P=0.003). The CMBs patients were more likely to be women (64.1% vs 52.2%; P=0.030), have hypertension (68.0% vs 52.7%; P=0.005), and carry ALDH2 *2 allele (P=0.020) than non-CMBs. No significant difference was observed for BMI, cigarette smoking, DM and hyperlipidemia between CMBs and non-CMBs.

We classified all subjects into two groups by genotype of ALDH2, i.e., ALDH2 *1/*1 (*1 allele group) vs. ALDH2 *1/*2 and *2/*2 (*2 allele group). As shown in Table 2, alcohol consumption was less frequent in ALDH2 *2 allele group (P<0.001), whereas hypertension (P=0.043) and CMBs (P=0.007), particularly deep/mixed CMBs (P=0.008), were more common in ALDH2 *1 allele group. No significant differences were found for other vascular risk factors such as age, sex, BMI, smoking, DM, and hyperlipidemia.

Table 3 shows the association between ALDH2 gene polymorphisms and CMBs. ALDH2 *1/*1 genotype was significantly associated with CMBs before (OR: 1.93, 95% CI: 1.21–3.06) and after adjusting for other vascular risk factors (OR: 1.87, 95% CI: 1.14–3.06). The association was more pronounced in deep/mixed CMBs (OR: 2.53, 95% CI: 1.28–5.03) than that in lobar CMBs (OR: 1.39, 95% CI: 0.72–2.70). Further analysis observed a significant interaction between ALDH2 genotype and sex or hypertension on CMBs (all P<0.05). ALDH2 genotype was also observed to have a borderline interaction with alcohol drinking (P=0.076) on CMBs after adjusting possible confounders. Stratified analyses by sex, alcohol drinking and hypertension status showed that ALDH2 *1/*1 genotype was significantly associated with CMBs only among men (OR: 4.51, 95% CI: 1.70–11.97), non-alcohol drinkers (OR: 1.76, 95% CI: 1.02–3.04; P=0.044) or hypertension patients (OR: 2.21, 95% CI: 1.17–4.17).

Discussion

The present study aimed to investigate the relationship between the polymorphisms of ALDH2 gene and CMBs. Based on the baseline data of TIS (phase I), we found that ALDH2 *1/*1 genotype was associated with great odds of CMBs in aged 55–65 years Han Chinese population, especially in men, non-drinkers or hypertension patients.

As a member of detoxifying enzymes ALDH family, ALDH2 plays an important role in the process of metabolizing acetaldehyde to acetic acid (27). ALDH2 *2 allele is the most common single point mutation related with reduce enzyme activity. The mutation is more prevalent in Asian population (28). Similar to this previous report, we found 41.4% participants of this study with ALDH2 *2 allele and 5.1% individuals were *2/*2 genotype. We also found that individuals with ALDH2 *2/*2 genotype were less likely to drink alcohol than those with *1/*1 genotype.

ALDH2 *2 allele showed strong protection against stroke in Han Chinese with a history of heavy drinking (14), and excessive ethanol consumption aggravates the ischemic brain injury by inhibiting ALDH2 gene function (29). Moreover, ALDH2 gene could clear 4-hydroxy-2-nonenal and down regulate aquaporin 4 to protect against stroke (30,31). Recently, several studies found that ALDH2 *2 allele acted as an independent risk factor for ischemic stroke and cerebral infarctions in Chinese (11-13). However, many of other studies reported that the *1 allele of ALDH2 appears to be a significant risk factor for intracranial vascular stenosis in ischemic stroke (16), multiple lacunar infarcts (15), and stroke (14,17) in Asian population, which were similar with our results. We found that ALDH2 *1/*1 genotype was positively associated with CMBs in Chinese elderly population. So far, there is no consistent conclusion

| Characteristics | *1/*1 (n=322) | *1/*2 and *2/*2 (n=227) | P value |
|-----------------|---------------|-------------------------|---------|
| Age, mean (SD), years | 59.36 (2.66)  | 58.94 (2.77)            | 0.075   |
| Women, n (%)     | 171 (53.1)    | 128 (56.4)              | 0.501   |
| BMI, mean (SD)   | 24.30 (3.43)  | 23.82 (3.12)            | 0.098   |
| Smoking, n (%)   | 108 (33.9)    | 83 (36.9)               | 0.523   |
| Alcohol drinking, n (%) | 121 (37.8) | 38 (16.9)              | <0.001  |
| Hypertension, n (%) | 191 (59.3) | 114 (50.2)              | 0.043   |
| Diabetes, n (%)  | 41 (12.7)     | 32 (14.1)               | 0.737   |
| Hyperlipidemia, n (%) | 157 (48.8) | 107 (47.1)              | 0.774   |
| CMBs, n (%)      | 73 (22.7)     | 30 (13.2)               | 0.007   |
| Deep/mixed (%)   | 42 (13.0)     | 13 (5.7)                | 0.008   |
| Lobar            | 31 (9.6)      | 17 (7.5)                | 0.471   |

Table 2 Basic characteristics of participants across different ALDH2 genotypes

5 subjects had missing data for smoking and drinking status. ALDH2, aldehyde dehydrogenase 2; SD, standard deviation; BMI, body mass index; CMBs, cerebral microbleeds.

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Table 3: Stratified analysis for ALDH2 as risk factor in participants with CMBs by location, sex, drinking and hypertension status

| ALDH2 (*1 vs. *2)     | Univariate model | Model 1 | Model 2 |
|-----------------------|------------------|---------|---------|
|                       | OR (95% CI)      | P value | OR (95% CI) | P value | OR (95% CI) | P value |
| Overall               | 1.93 (1.21–3.06) | 0.006   | 1.91 (1.17–3.12) | 0.009   | 1.87 (1.14–3.06) | 0.013   |
| CMBs location         |                  |         |         |         |         |         |
| Deep/mixed            | 2.56 (1.34–4.89) | 0.005   | 2.55 (1.29–5.05) | 0.007   | 2.53 (1.28–5.03) | 0.008   |
| Lobar                 | 1.44 (0.78–2.68) | 0.247   | 1.44 (0.75–2.78) | 0.273   | 1.39 (0.72–2.70) | 0.325   |
| Sex                   |                  |         |         |         |         |         |
| Men                   | 3.26 (1.37–7.75) | 0.008   | 4.43 (1.70–11.49) | 0.002   | 4.51 (1.70–11.97) | 0.003   |
| Women                 | 1.53 (0.87–2.71) | 0.140   | 1.29 (0.71–2.34) | 0.401   | 1.28 (0.70–2.34) | 0.429   |
| Alcohol consumption   |                  |         |         |         |         |         |
| Drinking              | 2.74 (0.77–9.69) | 0.118   | 2.20 (0.60–8.00) | 0.232   | 1.91 (0.51–7.12) | 0.334   |
| Non-drinking          | 1.94 (1.15–3.26) | 0.013   | 1.77 (1.03–3.06) | 0.040   | 1.76 (1.02–3.04) | 0.044   |
| Hypertension status   |                  |         |         |         |         |         |
| Hypertension          | 2.19 (1.20–4.01) | 0.011   | 2.21 (1.18–4.15) | 0.014   | 2.21 (1.17–4.17) | 0.014   |
| Non-hypertension      | 1.39 (0.66–2.93) | 0.393   | 1.51 (0.67–3.39) | 0.323   | 1.52 (0.67–3.43) | 0.318   |

Model 1 was adjusted for sex, age, BMI, smoking, and drinking; model 2 was additionally adjusted for hypertension, DM, and hyperlipidemia. ALDH2, aldehyde dehydrogenase 2; CMBs, cerebral microbleeds; OR, odds ratio; CI, confidence interval; BMI, body mass index; DM, diabetes mellitus.

about the association between polymorphisms of ALDH2 and stroke. Additionally, we did not find certain reports concentrating on the relationship between the ALDH2 and CMBs in other population.

Hypertension has been proven to be a definite risk factor for CMBs (4,5). As a multifactorial disease, the underlying pathogenesis of CMBs points to arteriolosclerosis and cerebral amyloid angiopathy, and is strongly associated with aging and hypertension (4). Previous studies found that deep/mixed CMBs were associated with hypertensive vasculopathy, while strictly lobar CMBs were related to cerebral amyloid angiopathy (4). We found a higher prevalence of hypertension in participants with ALDH2 *1/*1 genotype than that in ALDH2 *2 allele carriers (Table 2). Similarly, Wu et al. found that ALDH2 *1/*1 genotype was significantly associated with essential hypertension, especially in men in a case-control study in China and a meta-analysis Asia (32). Furthermore, a meta-analysis of genome-wide association studies found that variants in ALDH2 were associated with blood pressure in east Asians (33). In present study, we found that ALDH2 *1/*1 genotype is associated with greater odds of deep/mixed CMBs but not for lobar CMBs, which consistent with the CMBs location pathologies distribution. In addition, ALDH2 *1 allele was reported associated with intracranial vascular stenosis (16), atherosclerosis (34), and higher plasma homocysteine level (16) and so on, which are all the risk factors for cerebrovascular disease.

We found that the association between ALDH2 gene polymorphisms and CMBs was more pronounced in men, which is consistent with several previous studies. Nagasawa et al. (15) reported that ALDH2 *1/*1 genotype was significantly associated with multiple lacunar infarcts in Japanese men. ALDH2*2 variant allele was shown as an independent protective factor against high alcohol consumption in male stroke patients in China (14). Cheng et al. (35) found ALDH2 gene polymorphisms was a risk factor in modified ischemic stroke in Chinese men, but not in women. It is worth noting that ALDH2 *1/*1 was significantly associated with essential hypertension in men (32), which may be a potential mechanism for high risk of CMBs and stroke in men with ALDH2 *1/*1 genotype. Whether alcohol drinking is an independent risk factor for stroke is controversial (36). Heavy alcohol consumption can predict incident CMBs in deep region but not in lobar brain regions (37), and individuals with ALDH2 *1/*1 genotype tend to be alcohol
drinker, which might increase the risk for hypertension (38). However, we did not find significant relationship between alcohol drinking and CMBs in this study. We conducted stratified analysis and found that ALDH2 *1/*1 genotype was a risk factor for CMBs, especially among non-drinkers. In addition, we did not find significant association between alcohol drinking and hypertension in our participants. To some extent, these results suggest that ALDH2 *1/*1 genotype leading to hypertension might not entirely result from alcohol consumption. By stratification of the blood pressure, it showed that participants with ALDH2 *1/*1 genotype had an additive effect on CMBs in hypertensive patients. Therefore, the definite biological mechanisms underlying this relationship need further to investigate.

To our best knowledge, this is the first study to demonstrate that a polymorphism at ALDH2 was associated with CMBs in elderly population in China. However, several limitations should be acknowledged. First, selection bias could not be avoided in the present study, since our subjects were not selected randomly, but recruited on volunteering in rural China. Second, the sample size was relatively small, limiting statistical power to identify modifying effects of lower exposures in association of ALDH2 polymorphisms with CMBs. Additionally, evaluation of the association between ALDH2 polymorphisms and progression of CMBs (i.e., increase of CMBs number) would be informative in future perspective studies. Finally, we didn’t collect information on amount of alcohol drinking, and could not evaluate the possible different effects of low, medium or heavy alcohol drinking on CMBs.

Conclusions

In summary, the present study is the first to demonstrate that the ALDH2 *1/*1 genotype is positively associated with CMBs in rural Chinese population aged 55–65 years, particularly among men, non-alcohol drinkers or hypertension patients. A multi-center large scale prospective study is warranted to confirm our findings in the future.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Ethics Committee of the School of Life Sciences, Fudan University, Shanghai, China (institutional review board approval number: 469), and all participants gave written informed consent.

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### Table S1: Analysis for ALDH2 as risk factor in participants with CMBs

| ALDH2 (*1 vs. *2) | Univariate model | Model 1 | Model 2 |
|-------------------|------------------|---------|---------|
|                   | OR (95% CI)      | P value | OR (95% CI) | P value | OR (95% CI) | P value |
| rs671             | 1.93 (1.21–3.06) | 0.006   | 1.91 (1.17–3.12) | 0.009 | 1.87 (1.14–3.06) | 0.013 |
| rs2074356         | 2.13 (1.25–3.64) | 0.004   | 2.14 (1.23–3.72) | 0.005 | 2.09 (1.20–3.65) | 0.007 |
| rs2238151         | 1.31 (0.57–3.03) | 0.521   | 1.23 (0.53–2.87) | 0.628 | 1.31 (0.53–3.10) | 0.532 |
| rs4646777         | 0.76 (0.49–1.16) | 0.201   | 0.78 (0.50–1.21) | 0.265 | 0.78 (0.50–1.22) | 0.270 |

Model 1 was adjusted for sex, age, BMI, smoking, and drinking; model 2 was additionally adjusted for hypertension, DM, and hyperlipidemia. ALDH2, aldehyde dehydrogenase 2; CMBs, cerebral microbleeds; OR, odds ratio; CI, confidence interval; BMI, body mass index; DM, diabetes mellitus.