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The ACTB Variants and Alcohol Drinking Confer Joint Effect to Ischemic Stroke in Chinese Han Population

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Aim: β-actin (*ACTB*) participates in the vascular remodeling and contributes to the cardiovascular diseases. Herein, we investigated the associations of *ACTB* with hypertension and stroke.

Methods: Three single-nucleotide polymorphisms in *ACTB* were selected for genotyping in 2,012 hypertension cases and 2,210 controls. The associations of *ACTB* with hypertension and stroke were examined in another follow-up study. Logistic and Cox regression were performed in a case-control study and a follow-up study, respectively. Additive scale interaction was examined by calculating the relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP) and synergy index (SI). The multiplicative interaction hazard ratio was calculated by fitting the Cox regression model. *ACTB* mRNA in peripheral blood mononuclear cells was measured in ischemic stroke (IS) cases and in controls.

Results: The associations of rs852426 with hypertension and stroke had statistical significance in drinkers but not after Bonferroni correction. An additive interaction of rs852426 and drinking was observed for stroke incidence, the adjusted RERI was −0.907 ($p=4.108\times10^{-4}$), and the multiplicative interaction was still sound (HR=0.541, $p=0.048$). Furthermore, the significant interaction was further replicated in a nested case-control study. In the drinking population, the relative expression of *ACTB* mRNA in IS was lower (0.99±0.26) than that in controls (1.13±0.20), with a p value of 0.026.

Conclusions: *ACTB* rs852426 was significantly associated with alcohol consumption on stroke risk, and the expression of *ACTB* mRNA in IS who had a drinking habit was significantly down-regulated. This finding will provide a novel insight into the prevention of stroke.

Key words: *ACTB*, Alcohol drinking, Variant, Ischemic stroke, Interaction

Introduction

Hypertension is a global public-health challenge because of its high prevalence and driving role in stroke. Stroke has been a leading cause of death in recent years in China[1)]. The cerebrovascular remodeling induced by chronic hypertension promotes the development of stroke incidence. Although numerous established risk factors for hypertension and stroke have been highlighted, a particular genetic alteration or genotype combination and its interaction with environmental factors is also involved in the occur-

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rence of cardiovascular diseases (CVDs)[2, 3].

Abnormal vascular remodeling is a well-known risk factor for CVDs. The rho-associated kinase (ROCK) pathway regulates the intracellular actin cytoskeleton and initiates the remodeling of the vascular wall[4]. β-actin (encoded by *ACTB* , a downstream effector of ROCK) is widely distributed in all eukaryotic cells and underlies cell migration[5, 6]. As an abundant and highly conserved cytoskeleton structural protein, β-actin helps organize and maintain the cellular morphology by virtue of facilitating the processes of migration, division, growth, signaling and shaping the cytoskeleton[7, 8]. Marina Karakozova, *et al.*[9] found that arginylation of β-actin regulated actin filament properties and lamella formation in motile cells. Animal experiments have confirmed that increased actin polymerization and stress fiber formation would generate mechanical force to trigger vascular hypertrophy and hypertension[10]. *ACTB* gene hypomorphic mice died of uncharacterized development defects[11, 12], reflecting the essential role of *ACTB* in maintaining routine intracellular biological functions. However, there is no population-based study investigates the association of *ACTB* with hypertension.

The actin cytoskeleton has been validated its pivotal position in neuronal development and activity[13]. β-actin is also a signaling molecule. Studies have demonstrated that β-actin regulates endothelial nitric oxide synthase type 3 (eNOS-3) in platelets and vascular endothelial cells[14, 15]; these alterations probably contribute to vascular complications, atherosclerosis, and thrombotic diseases (such as ischemic stroke)[16]. Furthermore, a whole-exome sequencing study in syndromic brain malformations identified a novel *de novo* mutation (p.Gly268Arg) in *ACTB*. Determining whether *ACTB* variations were associated with stroke incidence would warrant a population-based study.

Given the abovementioned evidences, we speculate that β-actin might participate in the mechanism of vascular remodeling and thrombogenesis and subsequently mediate the pathogenesis of hypertension and stroke. Notably, Rothenfluh *et al.* and Offenhäuser *et al.* found that the dynamic regulation of the actin cytoskeleton in flies and mice, respectively, affects cellular and behavioral sensitivity to alcohol[17, 18]. Further research would be required to explore whether alcohol exposure in the nervous system would modify the biological activities of actin cytoskeleton.

The present study aimed to evaluate whether the variants of *ACTB* contribute to the genetic susceptibility to hypertension and stroke and to evaluate the light of gene-environment interactions with mRNA transcriptional analysis. These would provide a novel insight into our improved understanding of the genetic role of *ACTB* in hypertension and stroke.

Methods

Study Participants

In the case-control study, a total of 4,222 participants consisting of 2,012 hypertensive cases and 2,210 controls were drawn from the community hypertension survey (Yixing, Jiangsu Province, 2009)[19]. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg or associated with patients who were then receiving anti-hypertensive medication, and patients who had a clinical history of secondary hypertension were excluded.

A total of 4,128 participants from the community hypertension survey in 2009 were enrolled. Flow charts of the case-control and follow-up studies are outlined in **Supplementary Fig. 1**. During a median follow-up time of 5.01 years, 613 incident cases with hypertension and 183 incident cases with stroke including 171 ischemic stroke (IS) cases and 12 hemorrhagic stroke (HS) cases were recorded on a disease register and a report system. The demographic characteristics for follow-up study are displayed in **Supplementary Table 1**.

To access the disease status of subjects in the follow-up study, both concentrated and household surveys were conducted. Data collected from the local hospitals, centers for disease control and prevention (CDCs), community health service centers and a social security center were further inspected to reduce the information bias.

All participants were interviewed and underwent physical examinations and laboratory tests. The demographic characteristics of the participants were obtained by trained research staffs though a standard questionnaire, including data such as age, gender, smoking and drinking status. The weight, height and BP of the participants were measured as previously described[19].

Participants with a drinking habit were defined as those who currently or previously consumed alcoholic beverages at least 2 times per week for at least 6 months per year. A total of 901 subjects were identified with a drinking habit. The amount of alcoholic beverages that they consumed on a daily or weekly basis was divided into three categories: white spirits, beer and wine (assuming that the content of ethanol was 42% in white spirits, 3% in beer and 10% in wine). The amount of alcohol consumed was calculated in grams per day. According to the standard drink of 15 g of ethanol, participators were divided into the following categories: never, light-to-moderate

(women: ≤ 1.0 drink/day; men: ≤ 2.0 drink/day) and heavy drinking group (women: >1.0 drink/day; men: >2.0 drink/day). Besides, daily or weekly alcohol consumption data for 104 individuals with a drinking habit were missing. We grouped these subjects into the light-to-moderate drinking group out of conservative considerations. Finally, 3,321, 369 and 532 subjects were categorized as non-drinking, light-to-moderate drinking and heavy drinking, respectively.

A nested case-control study was conducted to validate the observed interactive associations of single-nucleotide polymorphisms (SNPs) and drinking with stroke. A total of 24,352 subjects from a community-based epidemiological survey were recruited in Nantong city, Jiangsu Province from 2007 to 2008. Over a course of 9 years of follow-up, 860 incident stroke cases including 821 IS cases and 39 HS cases were identified according to the records from the local hospitals and CDCs. And 1,960 age- (5-year-old group) and gender-matched controls were enrolled for the analysis (**Supplementary Table 2**).

The study was approved by the ethics committee of Nanjing Medical University and conducted according to the principles of the Declaration of Helsinki. The written informed consent was obtained from all subjects during epidemiological interviews.

SNP Selection

The *ACTB* gene locates on chromosome 7p22.1 (Gene ID: 60; NC_000007.14), spans 3.4 kbps and contains 6 exons. We searched the SNP-covered *ACTB* gene from the upstream 5 kb to the downstream 2 kb and selected tagging SNPs (tagSNPs) from the database of the Chinese Han population in Beijing, China of the International Hap MAP Project (HapMap Data Rel 24/phase II Nov08, on NCBI B36 assembly, dbSNPb126). All tagSNPs were selected with a minor allele frequency (MAF) ≥ 0.05 and linkage disequilibrium (LD) r^2 ≥ 0.8. We also applied a functional candidate strategy to select potential functional SNPs on the bioinformatics effect prediction website (SNPINFO, https://snpinfo.niehs.nih.gov/). Finally, rs852426 (T>C), rs852423 (A>G), and rs2966449 (T>C) were selected, and their specific biological information was summarized in **Supplementary Table 3**.

Blood Sampling and SNP Genotyping

Blood samples were donated by participants for genotyping with adding ethylenediamine tetraacetic acid (EDTA)-containing receptacles. After an overnight fasting (>10 h), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and glucose (GLU) were measured. DNA was extracted using a standard phenole-chloroform method and stored at −20℃. Genotyping was performed using the TaqMan allelic discrimination assay in 384-well plates on the platform of 7900HT real-time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA). The primers and probes were designed using Primer Express Oligo Design software ver. 2.0 (ABI PRISM). Genotyping results were determined using SDS 2.3 Allelic Discrimination Software (Applied Biosystems). Meanwhile, each plate included blank samples as negative controls for the genotyping quality confirmation. The successful call rates of SNPs rs852426, rs852423 and rs2966449 were 99.90%, 99.95% and 99.92%, respectively.

Determination of *ACTB* mRNA

The expression of *ACTB* mRNA (NCBI Reference Sequence: NM_001101.4) in peripheral blood mononuclear cells (PBMCs) was measured between IS cases and age- and gender- matched controls (72 vs. 72). All IS cases were enrolled from the People's Hospital of Yixing City, Department of Neurology.

Total RNA was isolated from PBMCs (100 μl) using an RNA blood kit (Cat#Yu-B02-1, Yuan Corp., Wuxi, China) according to the manual instructions. A total of 7.5 μl RNA was used for cDNA reversion. cDNA was synthesized using TAKARA reverse transcription kits (RR047A Takara PrimeScript™ RT reagent kit with gDNA Eraser, Japan). The primers used were designed by using Primer Premier 5.0 software, and the primer sequences were listed as follows: forward (5'-3'), TGACGTGGACATCCGCAAAG and reversed (5'-3'), CTGGAAGGTGGACAGCGAGG.

Quantitative real-time PCR (qPCR) was processed in a final volume of 10 μl (2 μl cDNA, 5 μl HieffTM qPCR SYBR® GEEN Master Mix, 0.2 μl of each primer and 5 μl RNase-free water) running in triplicate using the ABI RT-PCR 7900 system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Using the glyceraldehyde phosphate dehydrogenase (*GAPDH*) gene as internal control, amplification of the *ACTB* gene was performed in the same tube under following thermal cycling conditions: initial denaturation at 95℃ for 5 min, 95℃ for 10 s, 60℃ for 20 s and 72℃ for 20 s, 95℃ for 15 s, 60℃ for 1 min and then 95℃ for 15 s with 40 cycles. Melting curve includes one cycle with 95℃ for 15 s, 60℃ for 1 min and 95℃ for 15 s. The standard deviation (*SD*) of cycle threshold (CT) values among each duplicate sample was less than 0.5. The expression of *ACTB* mRNA was calculated with the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

Unpaired Student's *t*-test was used to test the differences in all the quantitative variables among groups presented as mean ± SD. The Hardy–Weinberg equilibrium (HWE) for genotype frequencies was estimated with a Fisher's exact test in controls. Chi-square (χ^2) test was performed to compare the allele and genotype frequency distributions. Multiple unconditional logistic regression analyses were applied to evaluate the genetic effects of screened SNPs on hypertension by calculating the odds ratio (OR) and its 95% confidence interval (CI). Cox regression was applied to estimate hazard ratios (HRs) and 95% CI in the follow-up study. The interaction of drinking and *ACTB* polymorphisms with stroke incidence was estimated. The additive interaction was displayed by calculating the relative excess risk due to interaction (RERI), attributable proportion (AP) due to interaction and synergy index (SI)[20]. The multiplicative interaction hazard ratio was calculated by fitting the Cox regression model. Stratification analyses by gender, age group, smoking and drinking status were further conducted in both case-control and follow-up studies. A logistic regression model was used to evaluate the genetic effects of SNPs on stroke for the nested case-control study. A two-tailed p value of 0.05 was defined as statistical significance. All statistical analyses were performed with SPSS version 25.0 (SPSS, Inc., Chicago, IL).

Results

Demographic Characteristics at Baseline

The demographic characteristics of participants were summarized in **Table 1**. The hypertensive cases were average 3.42 years older than the controls ($p < 0.001$), although an age-matched (5 years) method was used for the analysis. Subjects with hypertension had higher levels of BMI, TC, TG, LDL-C and GLU than those of controls ($p < 0.001$), whereas no significant differences in gender, HDL-C, smoking status and drinking status were observed ($p > 0.05$). Overall, these characteristics were adjusted as confounding factors to evaluate the association of *ACTB* with hypertension.

Association Analysis for the Case-Control Study of Hypertension

The distributions of rs852426, rs852423 and rs2966449 in controls were consistent with *HWE* ($p > 0.05$). No significant associations were observed between each of the three SNPs and hypertension in the whole population (**Supplementary Table 4**). Stratification analysis by drinking status indicated that drinkers with the rs852426 CC genotype had a higher risk of hypertension than that of TT/TC carriers; after adjustment for age, gender, BMI, GLU, HDL-C, LDL-C, TC, TG and smoking status, the OR (95% CI) was 1.971 (1.053–3.691) with a p value of 0.034 (**Supplementary Table 5**). However, the associations mentioned above were not statistically significant after Bonferroni correction.

Association Analysis for the Follow-Up Study

The incidences density for hypertension and stroke were $681.85/10^4$ person-years and $86.55/10^4$ person-years, respectively. No significant associations were observed between the SNPs and hypertension, nor was stroke incidence in the whole population (**Supplementary Table 6**). A further stratification analysis by gender, age, smoking status and drinking status was conducted (**Supplementary Table 7**). Males with rs852423 AG/GG genotypes had a higher incidence of hypertension compared to AA genotype carriers; after being adjusted for covariates, HR (95% CI) was 1.334 (1.044–1.705), with $p = 0.021$. In females, individuals with the rs852423 GG genotype presented a higher risk of hypertension when compared with AA/AG carriers, the HR (95% CI) was 1.466 (1.038–2.068), $p = 0.030$. In the < 55 years age group, the rs852423 variation increased the risk of hypertension ($p = 0.043$), and the adjusted HR (95% CI) was 1.261 (1.008–1.578). In the drinking subpopulation, rs852426 T > C variation had a significant protective effect on stroke, and the adjusted HR (95% CI) was 0.470 (0.227–0.975), with a p value of 0.042 (**Table 2**). These results were not valid after Bonferroni correction.

The Interaction Analysis between *ACTB* rs852426 and Drinking

The analysis stratified by never, light-to-moderate drinking and heavy drinking status indicated that the rs852426 variation had a protective effect on stroke in subjects with the light-to-moderate or heavy drinking habit, the HRs (95% CIs) were 0.523 (0.174–1.571) and 0.485 (0.176–1.336), although the p values did not reach statistical significance (p values were 0.248 and 0.162). The association of rs852426 with stroke incidence mentioned above had no heterogeneity between light-to-moderate drinking and heavy drinking groups ($Q = 0.010$, $p = 0.935$). Thus, we combined light-to-moderate drinking and heavy drinking groups when we assessed the interaction between rs852426 and drinking.

It is observed that rs852426 significantly interacted with drinking in stroke incidence. The additive interaction analysis indicated that the rs852426 and

Table 1. Demographic and clinical characteristics of the hypertension case-control study

Characteristics	Group	Hypertension $n=2,012$	Normotension $n=2,210$	t/χ^2	p
Age (year)		62.35 ± 10.73	58.93 ± 10.45	10.484	< 0.001
Gender (%)	Male	829 (41.20)	884 (40.00)	0.632	0.427
	Female	1,183 (58.80)	1,326 (60.00)		
SBP (mm Hg)		142.86 ± 14.30	124.24 ± 11.36	47.018	< 0.001
DBP (mm Hg)		87.53 ± 8.54	79.08 ± 6.51	36.369	< 0.001
BMI (kg/m^2)		24.76 ± 3.51	23.64 ± 3.20	10.844	< 0.001
TC (mmol/L)		4.94 ± 1.05	4.78 ± 1.01	4.574	< 0.001
TG (mmol/L)		1.86 ± 1.58	1.53 ± 1.21	7.618	< 0.001
HDL-C (mmol/L)		1.36 ± 0.33	1.36 ± 0.33	0.175	0.751
LDL-C (mmol/L)		2.80 ± 0.89	2.64 ± 0.73	6.227	< 0.001
GLU (mmol/L)		5.83 ± 2.05	5.46 ± 1.61	6.684	< 0.001
Smokers (%)	Yes	480 (23.86)	533 (24.12)	0.039	0.843
	No	1,532 (76.14)	1,677 (75.88)		
Drinkers (%)	Yes	424 (21.07)	477 (21.58)	0.163	0.686
	No	1,588 (78.93)	1,733 (78.42)		

BMI, body mass index; DBP, diastolic blood pressure; GLU, glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Table 2. Stratification analyses of rs852426 with hypertension and stroke in the follow-up study

End point	Stratum	Genotype	N	Person-years	Incidence density (/10^4 Person-years)	HR (95% CI)		
						Additive model	Dominant model	Recessive model
Hypertension	Drinking	TT	86	1193.82	720.38	1.123 (0.848–1.487)*	1.205 (0.863–1.683)*	0.863 (0.371–2.007)*
		TC	63	709.91	887.44	$p=0.418$	$p=0.272$	$p=0.733$
		CC	6	86.02	697.51			
	Non-drinking	TT	271	4284.35	632.53	1.147 (0.974–1.349)*	1.162 (0.962–1.403)*	1.236 (0.766–1.995)*
		TC	169	2460.48	686.86	$p=0.100$	$p=0.119$	$p=0.385$
		CC	18	260.57	690.79			
Stroke	Drinking	TT	29	2799.11	103.60	0.470 (0.227–0.975)#	0.473 (0.219–1.021)#	-
		TC	11	1643.04	66.95	$p=0.042$	$p=0.057$	-
		CC	0	233.57	-			
	Non-drinking	TT	77	10142.37	75.92	1.278 (0.969–1.687)#	1.320 (0.943–1.847)#	1.449 (0.705–2.980)#
		TC	58	5731.56	101.19	$p=0.083$	$p=0.106$	$p=0.313$
		CC	8	671.54	119.13			

HR, hazard ratio; CI, confidence interval
*: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status and T2DM.
#: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, T2DM and hypertension.

drinking had a negative interaction; the RERI (95% CI) was −0.705(−1.377, −0.237), and the p value was 3.164×10^{-3}. After being adjusted for covariates, the interaction was still sound; the RERI (95% CI) was −0.907 (−1.411, −0.404) with a p value of 4.108×10^{-4}. The multiplicative interaction was still sound, the HR (95% CI) for the multiplicative interaction term was 0.541 (0.291, 0.988), with $p=0.048$. The detailed results are displayed in **Table 3**.

Validation of the Association of rs852426 and Alcohol Consumption with Stroke

In the nested case-control study, the interaction between rs852426 additive model and alcohol consumption on stroke was further validated in Nantong population (**Supplementary Table 8**). Although the association of rs852426 and stroke did not reach statistical significance, significant additive interaction between drinking and rs852426 was observed in the nested case-control study. The RERI (95% CI) was

Table 3. Association of rs852426 and drinking with stroke in the follow-up study

Interaction		Model 1	Model 2
Drinking*rs852426			
	HR (95% CI)	0.618 (0.349, 1.093)	0.541 (0.291, 0.994)
	p	0.098	0.048
Drinking + rs852426			
RERI	HR (95% CI)	−0.705 (−1.377, −0.237)	−0.907 (−1.411, −0.404)
	p	3.164×10^{-3}	4.108×10^{-4}
AP		−0.994	−1.398
SI		0.704	−0.630

HR, hazard ratio; CI, confidence interval; RERI, relative excess risk due to interaction; AP, attributable proportion due to interaction; SI, synergy index
Model 1 did not adjust any covariates.
Model 2 adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, T2DM and hypertension.

Table 4. Association of rs852426 and drinking with stroke in the nested case-control study

Interaction		Model 1	Model 2
Drinking*rs852426			
	HR (95% CI)	0.721 (0.568, 0.915)	0.749 (0.575, 0.976)
	p	0.007	0.033
Drinking + rs852426			
RERI	HR (95% CI)	−0.435 (−0.803, −0.066)	-0.370 (−0.622, −0.118)
	p	0.021	3.945×10^{-3}
AP		−0.589	−0.509
SI		−1.520	−2.815

HR, hazard ratio; CI, interval confidence; RERI, relative excess risk due to interaction; AP, attributable proportion due to interaction; SI, synergy index
Model 1 did not adjust any covariates.
Model 2 adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, T2DM and hypertension.

−0.370 (−0.622, −0.118), with a *p* value of 3.945×10^{-3}, and the OR (95% CI) was 0.749 (0.575–0.976), with a *p* value of 0.033 for the multiplicative interaction (Table 4).

Comparison of *ACTB* mRNA Relative Expression Level between IS Cases and Controls

No significant difference of the *ACTB* mRNA relative expression level was observed between IS and controls (1.03 ± 0.28 vs. 1.10 ± 0.18, Supplementary Fig. 2). A further stratified analysis by drinking status indicated that in the drinking population, the expression of *ACTB* in IS was significantly lower (0.99 ± 0.26) than that in the controls (1.13 ± 0.20), with a *p* value of 0.026 (Fig. 1). However, there is no significant difference of the *ACTB* mRNA level among rs852426 genotypes in IS or in controls (data not provided).

Discussion

The current study evaluated the associations of *ACTB* polymorphisms with hypertension and stroke. The key findings indicated that rs852426 polymorphisms were significantly associated with the effect of alcohol consumption on stroke incidence. In the drinking population, it was first observed that the expression of *ACTB* in IS was significantly down-regulated.

Alcohol consumption is mostly taken as one of the covariates in numerous genetic association studies; thus, the molecular mechanism behind the alcoholic effect on stroke is easily neglected. Our novel finding indicated that the expression of *ACTB* was significantly down-regulated in IS who had a drinking habit. This might speculate that a drug that can up-regulate of *ACTB* expression will be a novel treatment method for the drinking population.

As generally regarded as a constitutive house-

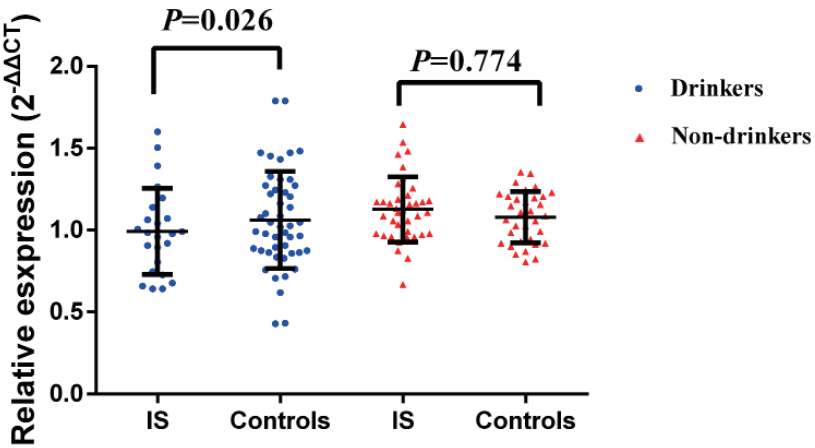

Fig. 1. The comparison of *ACTB* mRNA relative expression between IS cases and controls stratified by drinking status

The expression of *ACTB* in IS with a drinking habit was significantly lower (0.99 ± 0.26) than that in the controls (1.13 ± 0.20), with $p = 0.026$.

keeping gene, *ACTB* is an optimal reference gene for the quantitative reverse transcription polymerase chain reaction (RT-PCR) studies[21]. The obvious impact of alcohol on *ACTB* mRNA expression in IS dramatically indicates that selection of *ACTB* mRNA as a reference of a RT-PCR analysis for CVD may not be favorable.

Ongoing large GWAS have identified novel SNPs and pathogenic pathways[22], which could partly elucidate the mechanisms involved in the progression of stroke. The pathogenesis of stroke is affected by not only genetic factors but also interactions between genetics and environment risk factors (such as alcohol drinking, smoking, or physical activities). Light-to-moderate alcohol consumption has generally been accepted as a potential protective factor for stroke, whereas heavy alcohol consumption was recognized as a risk factor[23, 24]. Alcohol reduces thrombus formation by inhibiting platelet aggregation[25], antioxidant capacity and insulin sensitivity[26]; nonetheless, the biochemical mechanisms behind the cardiovascular beneficial effects of alcohol are still not fully elucidated.

The mutual protective effect between *ACTB* rs852426 polymorphisms and alcohol consumption on stroke provides a novel explanation for alcohol consumption attenuating the risk of stroke. Meanwhile, this interaction was further validated in a nested case-control study. To our knowledge, this study firstly observed the significant interaction of *ACTB* rs852426 and alcohol consumption on stroke. However, no significant difference of the *ACTB* mRNA level among rs852426 genotypes was observed; thus, elucidating the underlying modification of alcohol on *ACTB* mRNA expression in IS would warrant further biological function research.

The protective effect of *ACTB* rs852426 T>C variation on stroke incidence was observed both in the light-to-moderate and heavy drinking sub-population, although the *p* values did not reach statistical significance after multiple corrections. This could be explained by the limited stroke incidence in the follow-up study. The association of rs852426 with stroke has no heterogeneity between light-to-moderate and drinking heavy drinking groups. Given the universality of drinking in Chinese daily life, we may speculate that drinking is deprecated for the patients with the rs852426 TT genotype predisposed to stroke in clinic management.

A regional LD plot (http://www.broadinstitute.org/mpg/snap/ldplot.php) was depicted for rs852426 (**Supplementary Fig. 3**). Four loci (rs2537620, rs852432, rs2966450 and rs852446) were estimated near rs852426 with high LD ($r^2 > 0.8$), thus suggesting that a fine mapping for this region would be warranted.

Several limitations were presented in the current study. First, we selected candidate SNPs with the criteria of MAF ≥ 0.05 and could have missed the chance of evaluating rare variants at *ACTB* for hypertension and stroke. Second, all participants were from the south China Han population; thus, the subject diversity of varied cultures and lifestyles was limited. Third, the significance in results obtained was not consistent in follow-up studies. This may be attributed to the fact that the case-control studies have a larger sample size than the number of incident events in the follow-up studies. Fourth, the median follow-up time of this

study is 5.01 years, and only 613 hypertensions and 183 stroke incidences were recorded. The cumulative effect of *ACTB* on hypertension and stroke incidence should to be further confirmed by large-scale studies.

Conclusion

Our findings suggest that significant negative interaction between rs852426 and alcohol consumption could attenuate the stroke risk. The expression of *ACTB* in IS who had a drinking habit was significantly down-regulated compared to that in controls. It will be interesting to determine the potentially important role of alcohol consumption and *ACTB* polymorphisms in the prevention and treatment of stroke.

Conflict of Interests

There are no conflicts of interests.

Sources of Funding

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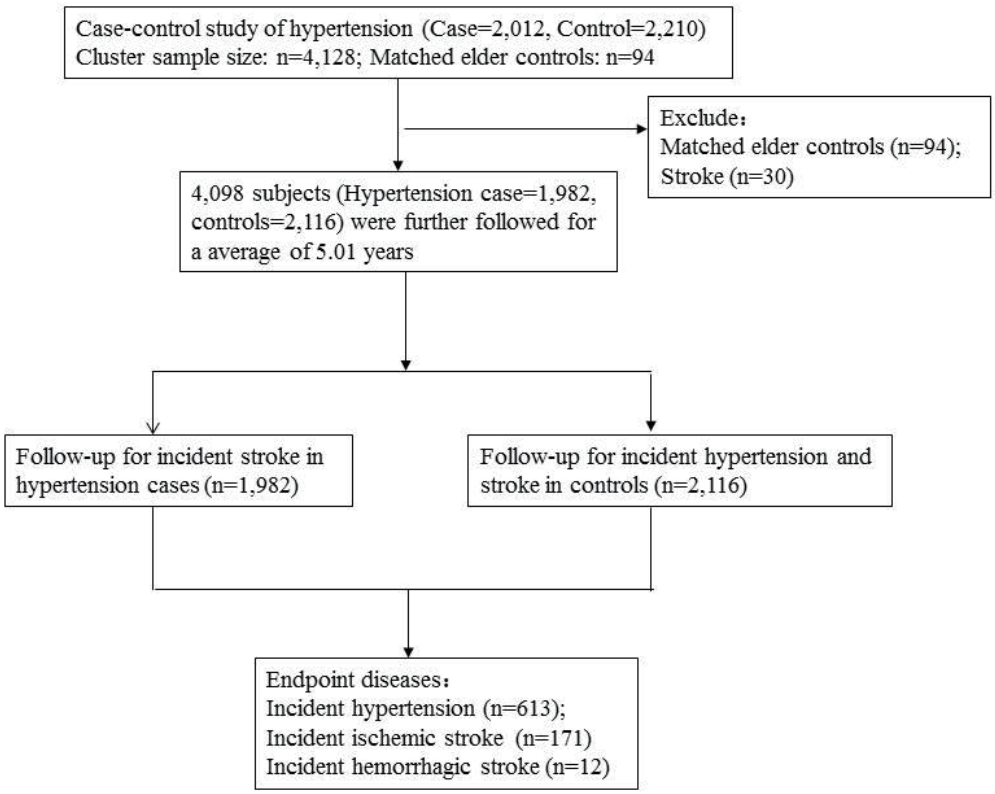


Supplementary Fig. 1. The flow chart of the case-control and follow-up studies

Inclusion of participants for evaluating the associations between *ACTB* and hypertension in the case-control study, and participants for the follow-up study. Matched elderly controls ($n=94$) and 30 hypertension cases with history of stroke were excluded in the follow-up study.

Supplementary Table 1. Demographic characteristics the follow-up study

Characteristics	Group	Follow-up study for hypertension $n=2116$	Follow-up study for stroke $n=4098$
Age (year)		58.41 ± 10.28	60.27 ± 10.67
Gender (%)	Male	853 (40.31%)	1660 (40.51%)
	Female	1263 (59.69%)	2438 (59.49%)
BMI (kg/m^2)		23.70 ± 3.21	22.21 ± 3.38
TC (mmol/L)		4.78 ± 1.01	4.85 ± 1.03
TG (mmol/L)		1.54 ± 1.21	1.69 ± 1.41
HDL-C (mmol/L)		1.37 ± 0.33	1.37 ± 0.33
LDL-C (mmol/L)		2.65 ± 0.72	2.72 ± 0.81
GLU (mmol/L)		5.47 ± 1.62	5.65 ± 1.86
Smokers (%)	Yes	525 (24.81%)	995 (24.28%)
	No	1591 (75.19%)	3103 (75.72%)
Drinkers (%)	Yes	468 (22.12%)	883 (21.55%)
	No	1648 (77.88%)	3215 (78.45%)
Hypertension (%)	Yes	-	1985 (48.43%)
	No	-	2113 (51.56%)
Diabetes (%)	T2DM	193 (9.12%)	461 (11.98%)
	IFG	418 (19.75%)	891 (21.74%)
	NGT	1505 (71.13%)	2746 (66.28%)

T2DM: type 2 diabetes mellitus; IFG: impaired fasting glucose; NGT: normal glucose tolerance

Supplementary Table 2. Demographic characteristics of the nested case-control study

Characteristics	Group	Stroke $n=860$	Controls $n=1960$	t/χ^2	P
Age (year)		63.24 ± 7.24	61.78 ± 4.94	6.341	< 0.001
Gender (%)	Male	353 (41.05%)	793 (40.46%)	0.085	0.77
	Female	507 (58.95%)	1167 (59.54%)		
BMI (kg/m^2)		24.06 ± 3.85	23.64 ± 4.42	2.363	0.018
TC (mmol/L)		4.32 ± 1.64	4.24 ± 0.92	1.545	0.122
TG (mmol/L)		1.63 ± 1.54	1.51 ± 1.38	2.076	0.038
HDL-C (mmol/L)		1.57 ± 0.41	1.63 ± 0.43	3.534	< 0.001
LDL-C (mmol/L)		2.08 ± 1.56	1.99 ± 0.72	2.057	0.04
GLU (mmol/L)		5.01 ± 2.61	5.51 ± 3.10	3.941	< 0.001
Smokers (%)	Yes	213 (24.77%)	445 (22.70%)	1.325	0.25
	No	647 (75.23%)	1515 (77.30%)		
Drinkers (%)	Yes	229 (26.63%)	583 (29.74%)	2.894	0.089
	No	631 (73.37%)	1377 (70.26%)		
Hypertension (%)	Yes	446 (51.86%)	701 (35.76%)	64.17	< 0.001
	No	414 (48.14%)	1259 (64.24%)		
Diabetes (%)	T2DM	99 (11.51%)	466 (23.77%)	70.727	< 0.001
	IFG	26 (3.02%)	110 (5.61%)		
	NGT	735 (85.47%)	1384 (70.62%)		

T2DM: type 2 diabetes mellitus; IFG: impaired fasting glucose; NGT: normal glucose tolerance

Supplementary Table 3. The biological information and function prediction of three tagSNPs of *ACTB* gene

No	SNP	Chromosome	Position	Allele	LDsnp	Pop/LD	TFBS	RegPotential	Conservation	Nearby Gene	Distance (bp)
1	rs852426	7	5532879	T/C	rs852426	1	--	0	0.002	MIRN589||ACTB	-30806||-433
2	rs852423	7	5534892	A/G	rs852423	1	--	0.266	0	ACTB	1580||1855
3	rs2966449	7	5538151	T/C	rs2966449	1	Msx-1	0.135	0	ACTB||FSCN1	-1404||-60829

TFBS, transcription factor binding site; RegPotential, regulatory potential score.

Supplementary Table 4. Association analyses of *ACTB* with hypertension in the case-control study

SNP	Group	WT/HT/MT	*OR* (95% *CI*)[a]			Allele gene		
			Additive model	Dominant model	Recessive model	Major/minor	*OR* (95% *CI*)/*P*[b]	*P*[c]
rs852426		TT/TC/CC				T/C		
	Case	1217/696/99	1.036 (0.929-1.156)	1.006 (0.884-1.144)	1.282 (0.944-1.740)	3130/894	0.963 (0.869-1.068)	0.478
	Control	1342/776/88	*P*=0.521	*P*=0.930	*P*=0.112	3460/952	*P*=0.239	
rs852423		AA/AG/GG				A/G		
	Case	993/809/209	1.040 (0.946-1.144)	1.049 (0.925-1.190)	1.063 (0.863-1.309)	2795/1227	0.961 (0.874-1.056)	0.406
	Control	1119/869/221	*P*=0.413	*P*=0.457	*P*=0.568	3107/1311	*P*=0.235	
rs2966449		TT/TC/CC				T/C		
	Case	1135/763/114	0.973 (0.877-1.080)	0.975 (0.858-1.107)	0.933 (0.713-1.220)	3033/991	1.005 (0.910-1.110)	0.924
	Control	1250/823/134	*P*=0.606	*P*=0.696	*P*=0.612	3323/1091	*P*=0.462	

WT, wild type, HT, heterozygote, MT, mutant type.
[a]: Adjusted for age, gender, body mass index (BMI), glucose (GLU), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), smoking and drinking status.
[b]: *P* value of χ^2 test for comparison of allele frequencies between hypertension case and control groups.
[c]: *P* value of χ^2 test for comparison of genotype between hypertension case and control groups.
The unsuccessful genotyped number of rs852426, rs852423 and rs2966449 was 4, 2 and 3, respectively.

Supplementary Table 5. Stratification analysis by age, gender, smoking and drinking status for the association of *ACTB* with hypertension in the case-control study

SNP	Stratum	Group	WT/HT/MT	*OR* (95% *CI*)		
				Additive model	Dominant model	Recessive model
rs852426			TT/TC/CC			
	Male	Case	509/273/47	0.957 (0.810-1.129)[a]	0.892 (0.729-1.090)[a]	1.275 (0.818-1.986)[a]
		Control	527/316/41	*P*=0.601	*P*=0.263	*P*=0.284
	Female	Case	708/423/52	1.115 (0.964-1.289)[a]	1.110 (0.937-1.315)[a]	1.316 (0.862-2.009)[a]
		Control	815/460/47	*P*=0.141	*P*=0.227	*P*=0.204
	<55 years	Case	339/172/23	0.931 (0.765-1.135)[b]	0.897 (0.711-1.130)[b]	1.067 (0.610-1.868)[b]
		Control	521/302/34	*P*=0.481	*P*=0.356	*P*=0.820
	≥ 55 years	Case	878/524/76	1.089 (0.955-1.243)[b]	1.063 (0.910-1.243)[b]	1.387 (0.959-2.008)[b]
		Control	821/474/54	*P*=0.202	*P*=0.441	*P*=0.083
	Smoking	Case	295/161/24	1.010 (0.805-1.267)[c]	0.931 (0.713-1.217)[c]	1.633 (0.854-3.123)[c]
		Control	324/191/18	*P*=0.932	*P*=0.603	*P*=0.138
	Non-smoking	Case	922/535/75	1.046 (0.924-1.185)[c]	1.030 (0.888-1.194)[c]	1.209 (0.854-1.711)[c]
		Control	1018/585/70	*P*=0.475	*P*=0.695	*P*=0.285
	Drinking	Case	256/141/27	1.098 (0.871-1.385)[d]	0.997 (0.753-1.322)[d]	1.971 (1.053-3.691)[d]
		Control	287/171/19	*P*=0.429	*P*=0.985	*P*=0.034
	Non-drinking	Case	961/555/72	1.026 (0.906-1.161)[d]	1.014 (0.877-1.174)[d]	1.132 (0.796-1.612)[d]
		Control	1055/605/69	*P*=0.685	*P*=0.847	*P*=0.490
rs852423			AA/AG/GG			
	Male	Case	410/330/88	0.999 (0.863-1.156)[a]	0.974 (0.800-1.185)[a]	1.066 (0.776-1.465)[a]
		Control	439/351/94	*P*=0.986	*P*=0.790	*P*=0.694
	Female	Case	583/479/121	1.079 (0.952-1.223)[a]	1.116 (0.946-1.316)[a]	1.069 (0.810-1.412)[a]
		Control	680/518/127	*P*=0.233	*P*=0.195	*P*=0.636
	<55 years	Case	266/219/48	1.000 (0.842-1.187)[b]	1.022 (0.816-1.280)[b]	0.936 (0.635-1.380)[b]
		Control	434/339/84	*P*=0.998	*P*=0.849	*P*=0.740
	≥ 55 years	Case	727/590/161	1.062 (0.948-1.191)[b]	1.066 (0.915-1.242)[b]	1.127 (0.878-1.447)[b]
		Control	685/530/137	*P*=0.298	*P*=0.413	*P*=0.349
	Smoking	Case	237/192/50	1.057 (0.869-1.285)[c]	1.012 (0.780-1.312)[c]	1.271 (0.824-1.960)[c]
		Control	272/211/50	*P*=0.579	*P*=0.930	*P*=0.278
	Non-smoking	Case	756/617/159	1.037 (0.930-1.156)[c]	1.059 (0.916-1.223)[c]	1.020 (0.804-1.295)[c]
		Control	847/658/171	*P*=0.514	*P*=0.441	*P*=0.870
	Drinking	Case	201/174/48	1.112 (0.906-1.364)[d]	1.102 (0.837-1.452)[d]	1.276 (0.822-1.981)[d]
		Control	235/192/50	*P*=0.309	*P*=0.489	*P*=0.277
	Non-drinking	Case	792/635/161	1.027 (0.923-1.144)[d]	1.043 (0.904-1.202)[d]	1.017 (0.801-1.290)[d]
		Control	884/677/171	*P*=0.622	*P*=0.567	*P*=0.892
rs2966449			TT/TC/CC			
	Male	Case	471/313/45	0.916 (0.779-1.076)[a]	0.921 (0.755-1.122)[a]	0.805 (0.531-1.220)[a]
		Control	489/336/58	*P*=0.285	*P*=0.412	*P*=0.306
	Female	Case	664/450/69	1.018 (0.888-1.167)[a]	1.017 (0.861-1.202)[a]	1.042 (0.731-1.484)[a]
		Control	761/487/76	*P*=0.800	*P*=0.840	*P*=0.820
	<55 years	Case	318/186/30	0.917 (0.760-1.106)[b]	0.871 (0.694-1.094)[b]	1.046 (0.644-1.697)[b]
		Control	483/324/48	*P*=0.364	*P*=0.234	*P*=0.856
	≥ 55 years	Case	817/577/84	0.988 (0.870-1.121)[b]	1.031 (0.883-1.203)[b]	0.894 (0.648-1.235)[b]
		Control	767/499/86	*P*=0.846	*P*=0.701	*P*=0.498
	Smoking	Case	269/188/23	1.010 (0.811-1.257)[c]	1.016 (0.782-1.320)[c]	0.987 (0.544-1.791)[c]
		Control	298/207/27	*P*=0.931	*P*=0.903	*P*=0.966
	Non-smoking	Case	866/575/91	0.964 (0.856-1.085)[c]	0.962 (0.831-1.113)[c]	0.930 (0.688-1.257)[c]
		Control	952/616/107	*P*=0.544	*P*=0.603	*P*=0.639
	Drinking	Case	231/167/26	1.022 (0.817-1.279)[d]	1.027 (0.780-1.354)[d]	1.027 (0.584-1.807)[d]
		Control	263/183/31	*P*=0.847	*P*=0.848	*P*=0.925
	Non-drinking	Case	904/596/88	0.966 (0.858-1.087)[d]	0.963 (0.834-1.112)[d]	0.938 (0.690-1.275)[d]
		Control	987/640/103	*P*=0.561	*P*=0.605	*P*=0.683

[a]: Adjusted for age, BMI, glucose, HDL-C, LDL-C, TC, TG, smoking and drinking status.
[b]: Adjusted for gender, BMI, glucose, HDL-C, LDL-C, TC, TG, smoking and drinking status.
[c]: Adjusted for age, gender, BMI, glucose, HDL-C, LDL-C, TC, TG and drinking status.
[d]: Adjusted for age, gender, BMI, glucose, HDL-C, LDL-C, TC, TG and smoking status.

Supplementary Table 6. Association analyses of *ACTB* with hypertension and stroke in the follow-up study

End Point	SNP	Genotype	N	Person-years	Incidence density (/10^4 Person-years)	*HR* (95% *CI*)		
						Additive model	Dominant model	Recessive model
Hypertension	rs852426	TT	357	5471.94	652.42	1.129 (0.984-1.297)[a]	1.167 (0.992-1.372)[a]	1.073 (0.712-1.619)[a]
		TC	232	3170.40	731.77	*P*=0.085	*P*=0.063	*P*=0.735
		CC	24	346.59	692.46			
	rs852423	AA	290	4526.55	640.66	1.120 (0.993-1.265)[a]	1.157 (0.986-1.357)[a]	1.154 (0.880-1.513)[a]
		AG	262	3587.49	730.32	*P*=0.066	*P*=0.073	*P*=0.299
		GG	59	874.90	674.36			
	rs2966449	TT	338	5043.27	670.20	1.039 (0.911-1.186)[a]	1.075 (0.916-1.262)[a]	0.923 (0.642-1.327)[a]
		TC	244	3396.07	718.48	*P*=0.568	*P*=0.374	*P*=0.665
		CC	31	546.53	567.21			
Stroke	rs852426	TT	106	12895.59	82.20	1.059 (0.821-1.366)[b]	1.074 (0.795-1.450)[b]	1.052 (0.514-2.151)[b]
		TC	69	7341.14	93.99	*P*=0.658	*P*=0.644	*P*=0.890
		CC	8	905.11	88.39			
	rs852423	AA	83	10663.54	77.84	1.170 (0.941-1.455)[b]	1.289 (0.958-1.735)[b]	1.080 (0.662-1.764)[b]
		AG	82	8366.72	98.01	*P*=0.157	*P*=0.093	*P*=0.757
		GG	18	2105.38	85.50			
	rs2966449	TT	98	11952.30	81.99	1.014 (0.793-1.298)[b]	1.064 (0.791-1.432)[b]	0.801 (0.391-1.640)[b]
		TC	77	7943.78	96.93	*P*=0.910	*P*=0.680	*P*=0.544
		CC	8	1239.61	64.54			

[a]: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, drinking status and T2DM.
[b]: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, drinking status, T2DM and hypertension.

Supplementary Table 7. Stratification analysis by age, gender, smoking and drinking status for the association of *ACTB* with hypertension and stroke in the follow-up study

End Point	SNP	Stratum	Genotype	N	Person-years	Incidence density (/10^4 Person-years)	*HR* (95% *CI*)		
							Additive model	Dominant model	Recessive model
Hypertension	rs852426	Male	TT	150	2154.16	696.33	1.106 (0.899-1.361)[a]	1.204 (0.940-1.542)[a]	0.754 (0.384-1.481)[a]
			TC	107	1269.52	842.84	*P*=0.341	*P*=0.142	*P*=0.413
			CC	9	159.13	565.58			
		Female	TT	207	3317.79	623.91	1.172 (0.969-1.418)[a]	1.148 (0.924-1.426)[a]	1.599 (0.942-2.714)[a]
			TC	125	1900.87	657.59	*P*=0.101	*P*=0.214	*P*=0.082
			CC	15	187.45	800.21			
		<55 years	TT	105	2225.96	471.71	1.120 (0.872-1.438)[a]	1.169 (0.868-1.575)[a]	1.011 (0.491-2.085)[a]
			TC	70	1331.86	525.58	*P*=0.376	*P*=0.304	*P*=0.975
			CC	8	157.06	509.36			
		≥ 55 years	TT	252	3245.98	776.34	1.166 (0.986-1.378)[a]	1.201 (0.989-1.459)[b]	1.158 (0.700-1.914)[b]
			TC	162	1838.53	881.14	*P*=0.072	*P*=0.065	*P*=0.568
			CC	16	189.53	844.19			
		Smoking	TT	85	1364.35	623.01	1.127 (0.856-1.485)[c]	1.248 (0.902-1.726)[c]	0.636 (0.231-1.751)[c]
			TC	70	787.22	889.21	*P*=0.394	*P*=0.181	*P*=0.381
			CC	4	83.65	478.18			
		Non-smoking	TT	272	4107.6	662.19	1.102 (0.935-1.299)[c]	1.090 (0.901-1.319)[c]	1.312 (0.833-2.065)[c]
			TC	162	2383.18	679.76	*P*=0.245	*P*=0.373	*P*=0.241
			CC	20	262.94	760.63			
		Drinking	TT	86	1193.82	720.38	1.130 (0.854-1.496)[d]	1.214 (0.870-1.693)[d]	0.872 (0.375-2.025)[d]
			TC	63	709.91	887.44	*P*=0.392	*P*=0.254	*P*=0.749
			CC	6	86.02	697.51			
		Non-drinking	TT	271	4284.35	632.53	1.144 (0.972-1.347)[d]	1.159 (0.960-1.399)[d]	1.233 (0.764-1.990)[d]
			TC	169	2460.48	686.86	*P*=0.105	*P*=0.126	*P*=0.390
			CC	18	260.57	690.79			
	rs852423	Male	AA	119	1768.20	673.00	1.141 (0.952-1.368)[a]	1.334 (1.044-1.705)[a]	0.841 (0.536-1.320)[a]
			AG	126	1448.11	870.10	*P*=0.153	*P*=0.021	*P*=0.451
			GG	21	366.50	572.99			
		Female	AA	171	2758.35	619.94	1.116 (0.948-1.315)[a]	1.056 (0.854-1.306)[a]	1.466 (1.038-2.068)[a]
			AG	137	2139.39	640.37	*P*=0.188	*P*=0.612	*P*=0.030
			GG	38	508.40	747.44			
		<55 years	AA	82	1859.30	441.03	1.261 (1.008-1.578)[b]	1.265 (0.941-1.701)[b]	1.581 (0.985-2.539)[b]
			AG	81	1503.42	538.77	*P*=0.043	*P*=0.120	*P*=0.058
			GG	20	352.17	567.91			
		≥ 55 years	AA	208	2667.25	779.83	1.072 (0.929-1.238)[b]	1.135 (0.938-1.373)[b]	0.984 (0.706-1.371)[b]
			AG	181	2084.08	868.49	*P*=0.340	*P*=1.135	*P*=0.924
			GG	39	522.74	746.07			
		Smoking	AA	69	1138.23	606.20	1.155 (0.906-1.473)[c]	1.267 (0.919-1.746)[c]	1.212 (0.890-1.650)[c]
			AG	77	886.57	868.51	*P*=0.246	*P*=0.148	*P*=0.222
			GG	13	210.41	617.84			
		Non-smoking	AA	221	3388.32	652.24	1.092 (0.948-1.259)[c]	1.088 (0.904-1.310)[c]	1.018 (0.572-1.814)[c]
			AG	186	2700.92	688.65	*P*=0.222	*P*=0.373	*P*=0.951
			GG	46	664.49	692.26			
		Drinking	AA	65	973.00	668.04	1.208 (0.950-1.535)[d]	1.392 (0.999-1.940)[d]	1.036 (0.600-1.789) [d]
			AG	75	811.04	924.74	*P*=0.124	*P*=0.051	*P*=0.898
			GG	15	205.47	730.03			

(Cont. Supplementary Table 7)

End Point	SNP	Stratum	Genotype	N	Person-years	Incidence density (/10^4 Person-years)	*HR* (95% *CI*)		
							Additive model	Dominant model	Recessive model
		Non-drinking	AA	225	3559.53	632.11	1.099 (0.953-1.267)[d]	1.099 (0.914-1.322)[d]	1.216 (0.887-1.663)[d]
			AG	188	2776.46	677.12	*P*=0.192	*P*=0.316	*P*=0.225
			GG	44	669.43	657.28			
	rs2966449	Male	TT	143	2002.44	714.13	1.072 (0.882-1.303)[a]	1.179 (0.923-1.506)[a]	0.769 (0.439-1.346)[a]
			TC	110	1343.78	818.59	*P*=0.486	*P*=0.187	*P*=0.357
			CC	13	233.50	556.75			
		Female	TT	195	3040.83	641.27	1.000 (0.835-1.199)[a]	0.981 (0.792-1.215)[a]	1.110 (0.686-1.795)[a]
			TC	134	2052.30	652.93	*P*=0.997	*P*=0.861	*P*=0.671
			CC	18	313.03	575.02			
		<55 years	TT	103	2070.92	497.36	1.020 (0.797-1.307)[b]	1.010 (0.750-1.359)[b]	1.100 (0.576-2.102)[b]
			TC	70	1426.06	490.86	*P*=0.873	*P*=0.950	*P*=0.772
			CC	10	209.84	476.55			
		≥ 55 years	TT	235	2972.36	790.62	1.055 (0.903-1.233)[b]	1.122 (0.927-1.358)[b]	0.842 (0.542-1.308)[b]
			TC	174	1970.02	883.24	*P*=0.501	*P*=0.238	*P*=0.444
			CC	21	336.69	623.72			
		Smoking	TT	85	1271.34	668.59	1.073 (0.825-1.395)[c]	1.152 (0.836-1.587)[c]	0.819 (0.378-1.775)[c]
			TC	67	837.37	800.12	*P*=0.598	*P*=0.387	*P*=0.613
			CC	7	123.41	567.21			
		Non-smoking	TT	253	3771.93	670.74	0.999 (0.855-1.168)[c]	1.001 (0.831-1.207)[c]	0.988 (0.652-1.495)[c]
			TC	177	2558.71	691.75	*P*=0.993	*P*=0.988	*P*=0.955
			CC	24	423.12	567.21			
		Drinking	TT	82	1094.57	749.15	1.080 (0.831-1.402)[d]	1.092 (0.786-1.516)[d]	1.128 (0.602-2.117)[d]
			TC	62	757.97	817.97	*P*=0.566	*P*=0.601	*P*=0.707
			CC	11	137.20	801.75			
		Non-drinking	TT	256	3954.92	647.30	1.013 (0.867-1.184)[d]	1.049 (0.868-1.260)[d]	0.865 (0.551-1.358)[d]
			TC	182	2638.10	689.89	*P*=0.870	*P*=0.636	*P*=0.529
			CC	20	409.32	488.62			
Stroke	rs852426	Male	TT	54	5271.40	102.44	0.908 (0.622-1.325)[a]	0.900 (0.583-1.387)[a]	0.860 (0.268-2.756)[a]
			TC	36	2981.01	120.76	*P*=0.617	*P*=0.632	*P*=0.799
			CC	3	420.76	71.30			
		Female	TT	52	7670.07	67.80	1.287 (0.902-1.836)[a]	1.340 (0.870-2.064)[a]	1.442 (0.561-3.705)[a]
			TC	33	4393.60	75.11	*P*=0.164	*P*=0.184	*P*=0.447
			CC	5	484.35	103.23			
		<55 years	TT	7	4766.93	14.68	1.308 (0.406-4.214)[b]	1.603 (0.417-6.156)[b]	-
			TC	5	2630.37	19.01	*P*=0.653	*P*=0.492	-
			CC	0	313.26	-			
		≥ 55 years	TT	99	8174.54	121.11	1.059 (0.815-1.376)[b]	1.068 (0.783-1.456)[b]	1.086 (0.529-2.229)[b]
			TC	64	4744.24	134.90	*P*=0.666	*P*=0.679	*P*=0.823
			CC	8	591.85	135.17			
		Smoking	TT	29	3202.08	90.57	0.734 (0.408-1.322)[c]	0.711 (0.369-1.372)[c]	0.627 (0.085-4.652)[c]
			TC	16	1824.74	87.68	*P*=0.303	*P*=0.309	*P*=0.648
			CC	1	212.43	47.07			
		Non-smoking	TT	77	9739.39	79.06	1.167 (0.873-1.558)[c]	1.193 (0.843-1.689)[c]	1.241 (0.573-2.686)[c]
			TC	53	5549.87	95.50	*P*=0.296	*P*=0.319	*P*=0.584
			CC	7	692.68	101.06			

(Cont. Supplementary Table 7)

End Point	SNP	Stratum	Genotype	N	Person-years	Incidence density (/10^4 Person-years)	*HR* (95% *CI*)		
							Additive model	Dominant model	Recessive model
		Drinking	TT	29	2799.11	103.60	0.470 (0.227-0.975)[d]	0.473 (0.219-1.020)[d]	-
			TC	11	1643.04	66.95	*P*=0.042	*P*=0.056	-
			CC	0	233.57	-			
		Non-drinking	TT	77	10142.37	75.92	1.278 (0.969-1.686)[d]	1.319 (0.943-1.847)[d]	1.448 (0.704-2.979)[d]
			TC	58	5731.56	101.19	*P*=0.083	*P*=0.106	*P*=0.314
			CC	8	671.54	119.13			
	rs852423	Male	AA	41	4335.75	94.56	1.179 (0.856-1.624)[a]	1.304 (0.851-2.000)[a]	1.055 (0.505-2.203)[a]
			AG	44	3432.82	128.17	*P*=0.313	*P*=0.223	*P*=0.884
			GG	8	898.40	89.05			
		Female	AA	42	6368.86	65.95	1.200 (0.884-1.628)[a]	1.314 (0.862-2.202)[a]	1.175 (0.600-2.300)[a]
			AG	38	4972.19	76.43	*P*=0.243	*P*=0.204	*P*=0.638
			GG	10	1206.99	82.85			
		<55 years	AA	6	3894.35	15.41	1.055 (0.364-3.057)[b]	1.543 (0.384-6.209)[b]	-
			AG	6	3088.90	19.42	*P*=0.921	*P*=0.541	-
			GG	0	721.09	-			
		≥ 55 years	AA	77	6810.25	113.06	1.182 (0.946-1.478)[b]	1.298 (0.956-1.763)[b]	1.120 (0.685-1.833)[b]
			AG	76	5316.10	142.96	*P*=0.142	*P*=0.095	*P*=0.651
			GG	18	1384.29	130.03			
		Smoking	AA	22	2649.69	83.03	0.893 (0.549-1.454)[c]	0.977 (0.528-1.809)[c]	0.522 (0.125-2.177)[c]
			AG	22	2068.29	106.37	*P*=0.650	*P*=0.941	*P*=0.372
			GG	2	515.05	38.83			
		Non-smoking	AA	61	8054.91	75.73	1.254 (0.978-1.607)[c]	1.370 (0.971-1.934)[c]	1.290 (0.762-2.184)[c]
			AG	60	6336.72	94.69	*P*=0.074	*P*=0.073	*P*=0.342
			GG	16	1590.34	100.61			
		Drinking	AA	19	2269.49	83.72	1.192 (0.718-1.979)[d]	1.499 (0.760-2.955)[d]	0.669 (0.159-2.814)[d]
			AG	19	1893.11	100.36	*P*=0.496	*P*=0.242	*P*=0.583
			GG	2	506.90	39.46			
		Non-drinking	AA	64	8435.11	75.87	1.207 (0.946-1.541)[d]	1.307 (0.934-1.829)[d]	1.218 (0.721-2.057)[d]
			AG	63	6511.89	96.75	*P*=0.130	*P*=0.119	*P*=0.460
			GG	16	1598.48	100.10			
	rs2966449	Male	TT	46	4881.39	94.24	1.325 (0.945-1.859)[a]	1.436 (0.940-2.194)[a]	1.308 (0.561-3.051)[a]
			TC	41	3256.39	125.91	*P*=0.103	*P*=0.094	*P*=0.534
			CC	6	529.21	113.38			
		Female	TT	52	7127.86	72.95	0.759 (0.521-1.104)[a]	0.794 (0.516-1.222)[a]	0.348 (0.083-1.464)[a]
			TC	36	4709.80	76.44	*P*=0.149	*P*=0.294	*P*=0.150
			CC	2	710.40	28.15			
		<55 years	TT	8	4438.70	18.02	0.706 (0.194-2.570)[b]	0.754 (0.186-3.050)[b]	-
			TC	4	2828.25	14.14	*P*=0.597	*P*=0.692	-
			CC	0	432.45	-			
		≥ 55 years	TT	90	7570.55	118.88	1.066 (0.828-1.371)[b]	1.131 (0.833-1.535)[b]	0.855 (0.417-1.755)[b]
			TC	73	5137.94	142.08	*P*=0.620	*P*=0.429	*P*=0.670
			CC	8	807.16	99.11			
		Smoking	TT	27	2950.55	91.51	0.804 (0.461-1.400)[c]	0.810 (0.432-1.516)[c]	0.548 (0.074-4.076)[c]
			TC	18	2014.60	89.35	*P*=0.441	*P*=0.509	*P*=0.557
			CC	1	267.90	37.33			

(Cont. Supplementary Table 7)

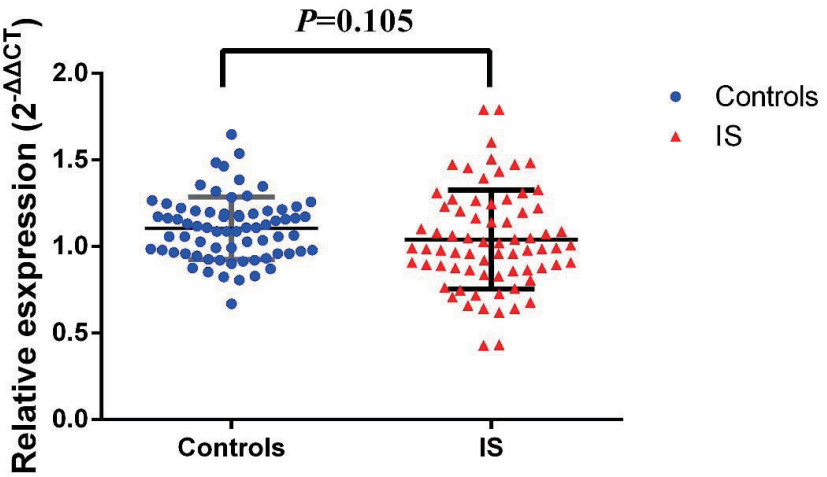
End Point	SNP	Stratum	Genotype	N	Person-years	Incidence density (/10^4 Person-years)	*HR* (95% *CI*)		
							Additive model	Dominant model	Recessive model
		Non-smoking	TT	71	9058.70	78.38	1.077 (0.813-1.428)[c]	1.143 (0.811-1.612)[c]	0.888 (0.408-1.933)[c]
			TC	59	5951.58	99.13	*P*=0.605	*P*=0.445	*P*=0.765
			CC	7	971.71	72.04			
		Drinking	TT	26	2558.70	101.61	0.768 (0.419-1.408)[d]	0.853 (0.434-1.675)[d]	-
			TC	14	1806.60	77.49	*P*=0.394	*P*=0.644	-
			CC	0	310.42	-			
		Non-drinking	TT	72	9450.55	76.19	1.096 (0.832-1.442)[d]	1.152 (0.823-1.612)[d]	0.969 (0.466-2.015)[d]
			TC	63	6159.58	102.28	*P*=0.515	*P*=0.410	*P*=0.933
			CC	8	929.19	86.10			

[a]: Adjusted for age, BMI, HDL-C, LDL-C, TC, TG, smoking status, drinking status and T2DM.
[b]: Adjusted for gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, drinking status and T2DM.
[c]: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, drinking status and T2DM.
[d]: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status and T2DM.
[e]: Adjusted for age, BMI, HDL-C, LDL-C, TC, TG, smoking status, drinking status, T2DM and hypertension.
[f]: Adjusted for gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, drinking status, T2DM and hypertension.
[g]: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, drinking status, T2DM and hypertension.
[h]: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, T2DM and hypertension

Supplementary Table 8. Association analyses of rs852426 with stroke in the nested case-control study

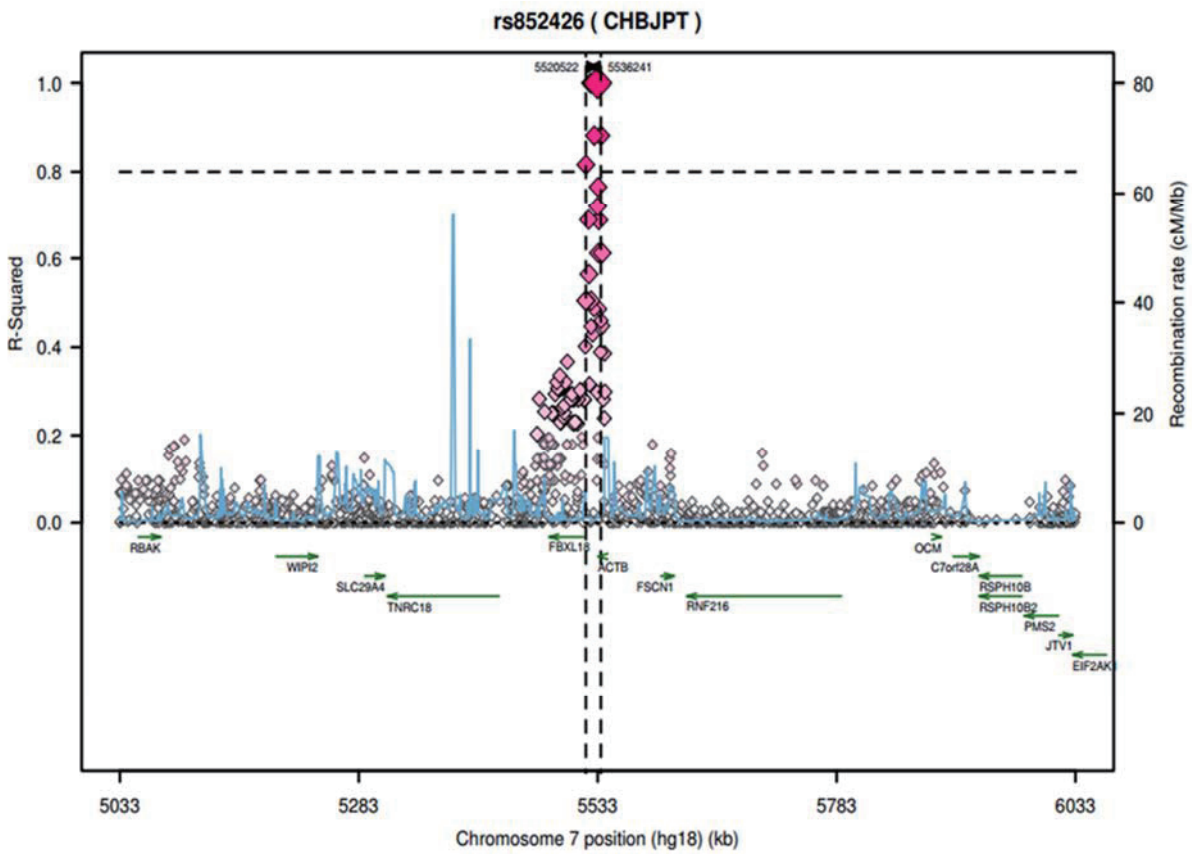
Stratification	Group	WT/HT/MT	*OR* (95% *CI*)		
			Additive model	Dominant model	Recessive model
Total Population		TT/TC/CC			
	Case	537/280/43	0.996 (0.859-1.154)[a]	1.040 (0.865-1.250)[a]	0.821 (0.555-1.215)[a]
	Control	1232/598/130	*P*=0.958[a]	*P*=0.678	*P*=0.324
Drinking		TT/TC/CC			
	Case	155/65/9	0.801 (0.591-1.087)[b]	0.807 (0.557-1.168)[b]	0.560 (0.236-1.327)[b]
	Control	352/195/36	*P*=0.154[b]	*P*=0.255	*P*=0.188
Non-drinking		TT/TC/CC			
	Case	383/214/34	1.080 (0.910-1.281)[b]	1.150 (0.927-1.427)[b]	0.926 (0.593-1.446)[b]
	Control	880/403/94	*P*=0.380[b]	*P*=0.204	*P*=0.735

WT, wild type; HT, heterozygote; MT, mutant type; OR, odds ratio; CI, interval confidence.
[a]: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, drinking status, T2DM and hypertension.
[b]: Adjusted for age, gender, BMI, HDL-C, LDL-C, TC, TG, smoking status, T2DM and hypertension.



Supplementary Fig. 2. The comparison of *ACTB* mRNA expression between IS and controls

No significant difference of *ACTB* mRNA was observed between IS and controls (1.03 ± 0.28 vs 1.10 ± 0.18, $P = 0.105$)



Supplementary Fig. 3. The regional LD Plot for rs852426, rs2537620, rs852432, rs2966450, and rs852446 were estimated loci near rs852426 with high LD ($r^2 > 0.8$)