Supplementary data 1

The experimental details of allele-specific PCR and restriction fragment length polymorphism analysis. Polymorphisms were genotyped using allele-specific PCR and restriction fragment length polymorphism analysis. PCR primers were synthesized by Sangon Biotech Co., Ltd.; the sequences are listed in Table SII. PCR conditions of ACE were as follows: 95˚C for 2 min, followed by 35 cycles at 95˚C for 30 sec, 55˚C for 30 sec, and 72˚C for 30 sec, final step was performed at 72˚C for 5 min. Amplified PCR products of intron 16 of ACE were separated on a 2% agarose gel (Bio-Rad Laboratories, Inc.) (Fig. 1). The presence of 191-bp fragments indicated the D allele, 480-bp fragments represented the I allele and 191- and 480-bp fragments represented the D/I allele. PCR conditions of β-Fg-455 were as follows: 94˚C for 2 min, followed by 35 cycles at 94˚C for 30 sec, 55˚C for 30 sec, and 72˚C for 80 sec, the final step was performed at 72˚C for 5 min. Amplification products of β-Fg-455 were digested by *Hae*III (FD0154; Thermo Fisher Scientific, Inc.) and then separated on a 2% agarose gel (Fig. 1). The presence of 343-, 383- and 575-bp fragments represented the GG allele; 343- and 958-bp fragments represented the AA allele, and 343-, 383-, 575- and 958-bp fragments represented the G/A allele. PCR conditions of β-Fg-148 were as follows: 94˚C for 2 min, followed by 35 cycles at 94˚C for 30 sec, 58.5˚C for 30 sec, and 72˚C for 30 sec, final step was performed at 72˚C for 5 min. Amplification products of β-Fg-148 were digested by *Hind*III (cat. no. FD0505; Thermo Fisher Scientific, Inc.) (Fig. 1). The presence of 100- and 200-bp fragments represented the CC allele, the presence of 300-bp fragments represented the TT allele and the presence of 100-, 200- and 300-bp fragments represented the T/C allele. PCR conditions of MTHFR were as follows: 94˚C for 2 min, followed by 35 cycles at 94˚C for 30 sec, 58˚C for 30 sec, and 72˚C for 30 sec, and the final step was performed at 72˚C for 5 min. Amplification products of MTHFR were digested by *Hinf*I (FD0804; Thermo Fisher Scientific, Inc.) (Fig. 1). The presence of 100- and 200-bp fragments represented the TT allele, 300-bp fragments represented the CC allele and 100-, 200- and 300-bp fragments represented the T/C allele. PCR conditions of PAI-1 were as follows: 94˚C for 2 min, followed by 35 cycles at 94˚C for 30 sec, 58.5˚C for 45 sec, and 72˚C for 30 sec, and the final step was performed at 72˚C for 5 min. PCR conditions of ApoE ε2,3,4 were as follows: 95˚C for 2 min, followed by 35 cycles at 95˚C for 30 sec, 60˚C for 45 sec, and 72˚C for 55 sec, and the final step was performed at 72˚C for 5 min.

Figure S1. ROC curve analysis of laboratory parameters to distinguish patients with ischemic stroke from normal controls. ROC, receiver operating characteristic; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; BMI, body mass index.

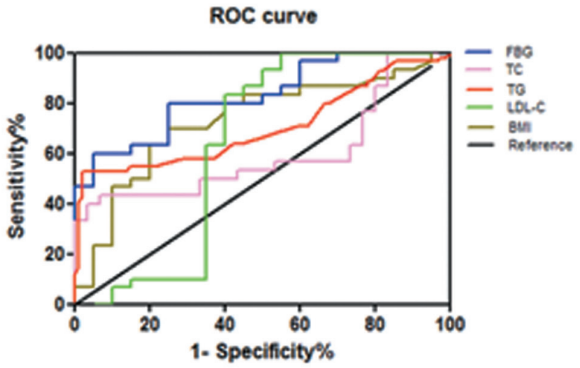

Table SI. Characteristics of the two groups at baseline.

Characteristic	Controls (n=36)	IS (n=62)	P-value
Age (years)	66 (41-89)	68 (43-91)	0.876
Sex (males)	152 (45%)	372 (48%)	0.453
Smoking	72 (22.3%)	198 (26.0%)	0.007
Drinking	54 (16.7%)	152 (19.9%)	0.018

IS, ischemic stroke.

Table SII. Sequences of PCR primers.

SNP	Sequences
ACE I/D	F: 5'-CTGGAGEGCCACTCCCATCCTTTCT-3'
	R: 5'-GACGTGGCCATCACATTCGTCAGAT-3'
MTHFR C677T	F: 5'-CTGTCATCCCTATTGGCAG-3'
	R: 5'-TGGGAAGAACTCAGCGAAC-3'
β-Fg-455 A/G	F: 5'-GGAATGCAATCTCTGCTACCT-3'
	R: 5'-TGTCGTTGACACCTTGGGAC-3'
β-Fg-148 T/C	F: 5'-CCTAACTTCCCATCATTTTGTC-3'
	R: 5'-ATGGTTTTAAGTTTGTGGAAGC-3'
PAI-1 4G/5G	F: 5'-AAGCTTTTACCATGGTAACCCCTGGT-3'
	R: 5'-TGCAGCCAGCCACGTGATTGTCTAG-3'
ApoE ε2,3,4	F: 5'-TCGGAACTGGAGGAACAAC-3'
	R: 5'-GCGTGAAACTTGGTGAATC-3'

F, forward; R, reverse; SNP, single nucleotide polymorphism; ACE, angiotensin-converting enzyme; MTHFR, methylene tetrahydrofolate reductase; β-Fg, β-fibrinogen; PAI, plasminogen activator inhibitor; ApoE, apolipoprotein E.

Table SIII. Association of BMI, HDL-C, ALT and AST with genotype frequencies of ACE I/D, MTHFR C677T, β-Fg-455A/G, β-Fg-148T/C, PAI-1 4G/5G and ApoE ε2-4 genes.

	BMI (kg/m^2)				HDL-C (mmol/l)		ALT (U/l)		AST (U/l)	
Item	≥28.00	24.00-27.99	18.50-23.99	<18.5	≥1.00	<1.00	≥40.00	<40.00	≥40.00	<40.00
ACE I/D										
II	11 (37.9)	17 (37.8)	132 (51.8)	5 (55.6)	144 (49.1)	21 (46.7)	11 (42.3)	154 (49.4)	13 (43.3)	152 (49.4)
ID	10 (34.5)	16 (35.6)	96 (37.8)	3 (33.3)	109 (37.2)	16 (35.6)	9 (34.6)	116 (37.2)	11 (36.7)	114 (37.0)
DD	8 (27.6)	12 (26.7)	27 (10.6)	1 (11.1)	40 (13.7)	8 (17.8)	6 (23.1)	42 (13.5)	6 (20.0)	42 (13.6)
χ^2/P-value		13.488/0.036			0.545/0.761		1.850/0.0.397		0.983/0.612	
MTHFR C677T										
CC	11 (37.9)	15 (33.3)	82 (32.2)	3 (33.3)	93 (31.7)	18 (40.0)	10 (38.5)	101 (34.2)	12 (40.0)	99 (32.1)
TC	12 (41.4)	20 (44.4)	133 (52.2)	4 (44.4)	148 (50.5)	21 (46.7)	12 (46.2)	157 (50.3)	13 (43.3)	156 (50.6)
TT	6 (20.7)	10 (22.2)	40 (15.7)	2 (22.2)	52 (17.7)	6 (13.3)	4 (15.4)	54 (17.3)	5 (16.7)	53 (17.2)
χ^2/P-value		2.610/0.856			1.369/0.504		0.406/0.816		0.811/0.667	
β-Fg-455A/G										
GG	14 (48.3)	22 (48.9)	166 (65.1)	7 (77.8)	180 (61.4)	29 (64.4)	15 (57.7)	194 (62.0)	18 (60.0)	191 (62.0)
GA	12 (41.4)	19 (42.2)	83 (32.5)	2 (22.2)	104 (35.5)	12 (26.7)	9 (34.6)	107 (34.3)	9 (30.0)	107 (34.7)
AA	3 (10.3)	4 (8.9)	6 (2.4)	0 (0.0)	9 (3.1)	4 (8.9)	2 (7.7)	11 (3.5)	3 (10.0)	10 (3.2)
χ^2/P-value		12.732/0.047			4.375/0.112		1.162/0.559		3.438/0.179	
β-Fg-148T/C										
CC	17 (58.6)	22 (48.9)	147 (57.6)	6 (66.7)	161 (54.9)	31 (68.9)	18 (69.2)	174 (55.8)	20 (66.7)	172 (55.8)
TC	10 (34.5)	18 (40.0)	93 (36.5)	3 (22.2)	111 (37.9)	12 (26.7)	7 (26.9)	116 (37.2)	8 (26.7)	115 (37.3)
TT	2 (6.9)	5 (11.1)	15 (5.9)	1 (11.1)	21 (7.2)	2 (4.4)	1 (3.8)	22 (7.1)	2 (6.7)	21 (6.8)
χ^2/P-value		3.177/0.786			3.108/0.211		1.822/0.402		1.420/0.492	
PAI-1 4G/5G										
4G4G	8 (27.6)	14 (31.1)	80 (31.4)	4 (44.4)	95 (32.4)	11 (24.4)	9 (34.6)	97 (31.1)	11 (36.7)	95 (30.8)
4G5G	11 (37.9)	16 (35.6)	136 (53.3)	4 (44.4)	140 (47.8)	27 (60.0)	13 (50.0)	154 (49.4)	15 (50.0)	152 (49.4)
5G5G	10 (34.5)	15 (33.3)	39 (15.3)	1 (11.1)	58 (19.8)	7 (15.6)	4 (15.4)	61 (19.6)	4 (13.3)	61 (19.8)
χ^2/P-value		14.510/0.024			2.335/0.311		0.314/0.855		0.893/0.640	
ApoE ε2-4										
E2/2	1 (3.4)	1 (2.2)	3 (1.2)	0 (0.0)	3 (1.0)	2 (4.4)	1 (3.8)	4 (1.3)	1 (3.3)	4 (1.3)
E2/3	6 (20.7)	8 (17.8)	36 (14.2)	1 (11.1)	42 (14.3)	9 (20.0)	4 (15.4)	47 (15.1)	6 (20.0)	45 (14.6)
E2/4	1 (3.4)	1 (2.2)	3 (1.2)	0 (0.0)	4 (1.4)	1 (2.2)	1 (3.8)	4 (1.3)	1 (3.3)	4 (1.3)
E3/3	16 (55.2)	24 (53.3)	180 (70.9)	7 (77.8)	203 (69.3)	25 (55.6)	14 (53.8)	214 (68.6)	15 (50.0)	213 (69.2)
E3/4	4 (13.8)	11 (24.4)	32 (12.6)	1 (11.1)	40 (13.7)	8 (17.8)	6 (23.1)	42 (13.5)	7 (23.3)	41 (13.3)
E4/4	1 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)
χ^2/P-value		20.490/0.154			2.820/0.324		4.554/0.473		5.574/0.350	

Values are expressed as n (%). BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ACE, angiotensin-converting enzyme; MTHFR, methylene tetrahydrofolate reductase; β-Fg, β-fibrinogen; PAI, plasminogen activator inhibitor; ApoE, apolipoprotein E.

Table SIV. Association of BMI, HDL-C, ALT and AST with allele frequencies of ACE I/D, MTHFR C677T, β-Fg-455A/G, β-Fg-148T/C, PAI-1 4G/5G and ApoE ε2-4 genes.

	BMI (kg/m^2)				HDL-C (mmol/l)		ALT (U/l)		AST (U/l)	
Item	≥28.00	24.00-27.99	18.50-23.99	<18.5	≥0.10	<0.10	≥40.00	<40.00	≥40.00	<40.00
ACE I/D										
I	32 (55.2)	50 (55.6)	360 (70.6)	13 (72.2)	397 (67.7)	58 (64.4)	31 (59.6)	424 (67.9)	37 (61.7)	418 (67.9)
D	26 (44.8)	40 (44.4)	150 (29.4)	5 (27.8)	189 (32.3)	32 (35.6)	21 (40.4)	200 (32.1)	23 (38.3)	198 (32.1)
χ^2/P-value	12.222/0.007				0.387/0.534		1.515/0.218		0.952/0.329	
MTHFR C677T										
C	34 (58.6)	50 (55.6)	297 (58.2)	10 (55.6)	334 (57.0)	57 (63.3)	32 (61.5)	359 (57.5)	37 (61.7)	354 (57.5)
T	24 (41.4)	40 (44.4)	213 (41.8)	8 (44.4)	252 (43.0)	33 (36.7)	20 (38.5)	265 (42.5)	23 (38.3)	262 (42.5)
χ^2/P-value	0.278/0.964				1.285/0.257		0.316/0.574		0.395/0.530	
β-Fg-455A/G										
G	40 (69.0)	65 (72.2)	415 (81.4)	14 (77.8)	464 (79.2)	70 (77.8)	39 (75.0)	495 (79.3)	45 (75.0)	489 (79.4)
A	18 (31.0)	25 (27.8)	95 (18.6)	4 (22.2)	122 (20.8)	20 (22.2)	13 (25.0)	129 (20.7)	15 (25.0)	127 (20.6)
χ^2/P-value	7.757/0.051				0.093/0.761		0.542/0.462		0.633/0.426	
β-Fg-148T/C										
C	44 (75.9)	62 (68.9)	387 (75.9)	14 (77.8)	433 (73.9)	74 (82.2)	43 (82.7)	464 (74.4)	48 (80.0)	459 (74.5)
T	14 (24.1)	28 (31.1)	123 (24.1)	4 (22.2)	153 (26.1)	16 (17.8)	9 (17.3)	160 (25.6)	12 (20.0)	157 (25.5)
χ^2/P-value	2.101/0.552				2.888/0.089		1.778/0.182		0.878/0.349	
PAI-1 4G/5G										
4G	27 (46.6)	44 (48.9)	296 (58.0)	12 (66.7)	330 (56.3)	49 (54.4)	31 (59.6)	348 (55.8)	37 (61.7)	342 (55.5)
5G	31 (53.4)	46 (51.1)	214 (42.0)	6 (33.3)	256 (43.7)	41 (45.6)	21 (40.4)	276 (44.2)	23 (38.3)	274 (44.5)
χ^2/P-value	5.641/0.130				0.111/0.739		0.288/0.591		0.839/0.360	
ApoE ε2-4										
E2	9 (15.5)	11 (12.2)	45 (8.8)	1 (5.6)	52 (8.9)	14 (15.6)	7 (13.5)	59 (9.5)	9 (15.0)	57 (9.3)
E3	42 (72.4)	67 (74.4)	430 (84.3)	16 (88.9)	488 (83.3)	67 (74.4)	38 (73.1)	517 (82.9)	43 (71.7)	512 (83.1)
E4	7 (12.1)	12 (13.3)	35 (6.9)	1 (5.6)	46 (7.8)	9 (10.0)	7 (13.5)	48 (7.7)	8 (13.3)	47 (7.6)
χ^2/P-value	10.277/0.113				4.752/0.093		3.311/0.191		4.908/0.086	

Values are expressed as n (%). BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ACE, angiotensin-converting enzyme; MTHFR, methylene tetrahydrofolate reductase; β-Fg, β-fibrinogen; PAI, plasminogen activator inhibitor; ApoE, apolipoprotein E.

Table SV. Prediction of ischemic stroke by the BMI and the levels of HDL-C, ALT and AST.

	Model 1				Model 2			
		95% CI				95% CI		
Factor	OR	Lower	Upper	P-value	OR	Lower	Upper	P-value
HDL-C (mmol/l)	0.27	0.08	0.92	0.038	0.32	0.15	0.67	0.002
BMI (kg/m^2)	2.26	1.35	2.79	<0.001	2.35	1.42	3.60	<0.001
ALT (U/l)	0.77	0.08	8.41	0.830	0.97	0.15	6.72	0.976
AST (U/l)	0.53	0.05	7.16	0.629	0.59	0.08	4.80	0.619

Model 1 is unadjusted and model 2 is adjusted for age and sex. BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; OR, odds ratio.

Table SVI. ROC curve analysis of BMI for ischemic stroke.

Item	Cut-off point	Specificity	Sensitivity	AUC	95% CI	
					Lower	Upper
BMI (kg/m^2)	23.12	0.877	0.591	0.735	0.685	0.786

ROC, receiver operating characteristic; AUC, area under the ROC curve; BMI, body mass index.