Original Article

Association between *ADAM17* Promoter Polymorphisms and Ischemic Stroke in a Chinese Population

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Aim: Stroke is a leading cause of death and disability worldwide. Most ischemic strokes (IS) are caused by atherosclerosis. Recently, the pivotal role of *ADAM17* in atherosclerosis has been thoroughly addressed. However, the association between *ADAM17* and IS has not yet been thoroughly explored. The present study therefore aimed to investigate the association between disintegrin and metalloproteinase 17 (*ADAM17*) gene polymorphisms and the risk of ischemic stroke (IS).

Methods: The associations between five *ADAM17* promoter polymorphisms and the risk of IS were assessed in 342 patients with IS and 296 age-matched healthy individuals in a case-control study.

Results: The allele and genotype frequencies of the *ADAM17* polymorphisms (rs11684747, rs11689958, rs12692386, rs55790676 and rs1524668) did not differ significantly between the IS patients and healthy control group subjects. In addition, no significant associations were detected between the *ADAM17* haplotypes and IS. The mean intima-media thickness in the IS patients was not associated with the *ADAM17* polymorphisms. When the IS patients were stratified according to their OSCP classification, the genotype frequencies of the *ADAM17*-rs1524668 polymorphism exhibited a significant association with the PACI subtype of IS. Moreover, the *ADAM17*-rs12692386 A>G polymorphism was found to be associated with a higher *ADAM17* mRNA expression.

Conclusions: The SNPs in the *ADAM17* promoter region do not appear to be major contributors to the pathogenesis of IS. However, the rs12692386 G *ADAM17* allele is correlated with a higher expression of *ADAM17* mRNA, which may play a role in increasing inflammation in IS patients. Furthermore, the *ADAM17*-rs1524668 polymorphism is linked to a higher risk of PACI-type stroke, confirming the role of *ADAM17* in the pathophysiology of PACI, with potentially important therapeutic implications.

J Atheroscler Thromb, 2014; 21:878-893.

Key words: *ADAM17*, Ischemic stroke, Polymorphism

Introduction

Stroke is the leading cause of long-term disability

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Received: October 25, 2013

Accepted for publication: March 5, 2014

and the third most common cause of death in many developed and developing countries. Approximately 80% of stroke cases involve ischemic stroke (IS)¹, which is primarily caused by atherosclerosis, a chronic inflammatory disease of the vessel wall². *ADAM* (a disintegrin and metalloproteinase) genes, which are characterized by their metalloproteinase domain and disintegrin-cysteine rich region, are essential for a number of biological processes, including inflammation, cell proliferation and migration, immunity and

angiogenesis³). Recently, ADAMs have been identified to be molecular scissors that cleave various substrates implicated in the processes of angiogenesis and inflammation that occur during atherosclerosis⁴). In addition, the dysregulation of ADAMs in the vasculature suggests their crucial roles in vascular biology⁵).

Among the ADAM family members, ADAM17 is the most relevant protease associated with IS. It is responsible for the generation of soluble tumor necrosis factor alpha (TNF- α), and its role in atherosclerosis has repeatedly been reported^{3, 5}). Additionally, ADAM17 has been documented to be capable of cleaving several other cell surface molecules, such as IL-6R, ICAM-1, VCAM-1, L-selectin, CX3CL and HB-EGF, which are considered to be involved in developmental, inflammatory and regenerative processes in the vasculature⁵). Several studies have clearly indicated an association between the ADAM17 expression and atherosclerosis. For example, the ADAM17 expression is upregulated in vessel wall cells, especially in macrophages present in advanced atherosclerotic plaques⁶). In patients with myocardial infarction, an increased expression of ADAM17 has been detected in ruptured coronary plaques⁷). In addition, microparticles released from atherosclerotic lesions harbor active ADAMs and significantly increase the shedding of TNF- α ⁸), while an atherosclerosis quantitative trait locus test recently identified *ADAM17* promoter variations that affect the mRNA expression *in vivo*, implicating *ADAM17* as a candidate gene causing susceptibility to atherosclerosis⁹). These results collectively demonstrate that ADAM17 plays an important role in the pathogenesis of IS.

The *ADAM17* gene is located on chromosome 2 and consists of 19 exons and 18 introns. One study reported that the C-154A and Ser747Leu SNPs of the *ADAM17* gene are associated with the TNF- α plasma level and risk of cardiovascular death, respectively¹⁰). Other research has indicated that two *ADAM17* polymorphisms, rs11684747 and rs10495563, are associated with obesity and insulin resistance-related phenotypes (higher insulin and lower HDL-C concentrations)¹¹). However, the associations between ADAM17 polymorphisms and IS have not yet been explored. Hence, the purpose of the present study was to: (1) investigate the associations between *ADAM17* polymorphisms and IS and (2) assess the relationships between *ADAM17* polymorphisms and the ADAM17 mRNA expression.

Materials and Methods

Subjects

A total of 342 ischemic stroke patients (mean age, 70.30 ± 10.75 years) were recruited from the First Affiliated Hospital of Guangdong Medical College between 2012 and 2013. An IS diagnosis was established based on the patient's clinical signs and symptoms. All patients underwent computed tomography (CT) scans and/or magnetic resonance imaging (MRI) as well as standardized blood tests. Based on the clinical manifestations and neuroimaging data, two neurologists classified the ischemic strokes into four subtypes using either the Trial of Org 10172 in Acute Stroke Treatment Criteria (TOAST)¹²) or Oxfordshire Community Stroke Project (OCSP) classification¹³). Patients with a history of transient ischemic attacks, cardiogenic cerebral infarction, cerebral hemorrhage, coronary artery disease, autoimmune disease, systemic inflammatory disease, blood diseases or malignant tumors were excluded from the study. One subject diagnosed with stroke was also excluded. The healthy control group consisted of 296 individuals (70.41 ± 8.57 years) recruited from the Health Examination Center of the Affiliated Hospital of Guangdong Medical College during the same time period, and the control patients were comparable to the IS subjects in terms of age, sex and race. The control subjects did not have a recent history of cerebrovascular disease or myocardial infarction. The same exclusion criteria were used as above. Smokers were defined as patients who were currently smoking at the time of the study, and hypertension was defined as a systolic pressure of >140 mm Hg and diastolic pressure of >90 mm Hg on more than one occasion and/or the current use of hypertensive medications. Diabetes mellitus was defined as a fasting plasma glucose level of >126 mg/dL (7.0 mmol/L) and/or the use of diabetic medications. The study was approved by the Ethics Committee of Guangdong Medical College. Written informed consent was obtained from each participant prior to study enrollment.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the EZ-10 Spin Column Whole Blood Genomic DNA Isolation Kit (Sangon Biotech®, Shanghai, China) according to the manufacturer's instructions. The DNA purity and concentration were determined using UV spectrophotometry measuring the absorbance at 260 and 280 nm with a Synergy™ 4 Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA). Five *ADAM17* SNPs (shown in **Supple-**

mentary Fig. 1) were selected based on previously published results¹⁴. The selected *ADAM17* SNPs were genotyped using the SNaPshot Multiplex Kit (Applied Biosystems Co., Ltd., Foster City, CA, USA), and the primers for PCR amplification and SNaPshot extension were designed according to the GenBank database. The primers used in the SNaPshot were as follows: s55790676_r s12692386F, GGCCTAGCCCCTCAATCCTCTT; rs55790676_r s12692386R, GACTGGGTGGAGTTGGGACTCA; rs116_152_116F, AAAAACGTTGGGTACATGTTGCTG rs116_152_116R, GAGCAAGGCACTGTACAAGAGCTG; rs55790676SF, TTT-TTTTTTTTTTTTTTGGCCGCTACTGGGAAGATTCTACC; rs12692386SR, TTTTTTTGGTAACGCCACCTGCACTTC; rs11684747SF, TTTTTTTACCGGACCTGGTCTGTACATCTGA; rs1524668SF, TTTTTTTTTTTTTTTTTTGGTTTCTTTTCTGAACATCCAGTCACCATA; and rs11689958SR, TTTTTTTTTTTTTTTTACAAGAGCTGTTGGGGATACCAAATAATA. The SNaPshot reactions (10 μ L final volume) contained SNaPshot Multiplex Ready Reaction Mix (5 μ L), primer mix (final concentration, 0.5 μ mol/L) and templates (4 μ L). The cycling program consisted of 28 cycles of 96°C for 10 seconds, 50°C for five seconds and 60°C for 30 seconds. Following amplification and purification, the PCR products were mixed and used as the template in the SNaPshot extension reaction. The extension products were purified using 15 minutes of incubation with 1 U shrimp alkaline phosphatase (Takara, Otsu, Shiga, Japan) at 37°C followed by 15 minutes of incubation at 80°C to inactivate the enzyme. The PCR products were then verified by electrophoresis. The quality of the genotyping data was analyzed using the GeneMapper 4.0 software program (Applied Biosystems Co., Ltd., Foster City, CA, USA). Ten percent of the samples were randomly selected to be sequenced.

Isolation of Mononuclear Cells

Peripheral blood mononuclear cells (PBMCs) were isolated via density gradient centrifugation using Lymphoprep™ (Axis-Shield, Oslo, Norway). Briefly, a blood sample was mixed with an equal volume of 0.9% NaCl. The diluted blood was then slowly added to layer of blood in a Ficoll premium solution. Then, the tubes were centrifuged at 800×g for 30 minutes at room temperature. Following centrifugation, the mononuclear cells formed a distinct band at the medium interface. The mononuclear cells were then transferred to new tubes and washed twice with 0.9% NaCl. All of the tubes were subsequently centrifuged at 250×g for 10 minutes and stored at -80°C until use.

RNA Extraction

Total cellular RNA was extracted from peripheral blood mononuclear cells using the RNeasy pure Blood Kit (TianGen Biotech, Beijing, China) according to the manufacturer's instructions. Briefly, the mononuclear cells were pelleted and lysed using TRIzol reagent. The lysate was then centrifuged at 13,400×g for two minutes, and the aqueous phase was transferred to a fresh micro-centrifuge tube and precipitated with ethanol. Next, the sample was bound to the silanized surface of the spin column.

Contaminating genomic DNA was eliminated via RNase-free DNase I digestion applied to the silica membrane. The bound RNA was further purified with washing and eluted from the membrane into water, and the RNA sample integrity was determined using agarose gel electrophoresis. The RNA samples were then stored at -80°C until use.

Real-Time PCR

The isolated RNA was transcribed into cDNA using the cDNA Synthesis Kit RevertAid (Thermo) according to the manufacturer's instructions. Quantitative real-time PCR was performed using the SYBR Green RT-PCR Kit (Takara). The real-time RT-PCR primers for both human GAPDH and *ADAM17* coding sequences were designed using the Primer Premier 5 software program (Applied Biosystems). The primer sequences used were as follows: *ADAM17* sense primer, CTGTGGTGCAAAAGCAGAAA; *ADAM17* anti-sense primer, TGCCAAATGCCTCATATTCA; GAPDH sense primer, GAAGGGCTCATGACCACAGTCCAT; and GAPDH anti-sense primer, TCATTGTCGTACCAGGAAATGAGCTT. The *ADAM17* and GAPDH mRNA levels were measured in triplicate, and the relative mRNA quantity was determined according to the $2^{-\Delta\Delta C_t}$ method and normalized with respect to that of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Real-time PCR was performed using a LightCycler® 480 sequence detector system (Roche Applied Sciences). The following PCR conditions were used over 40 cycles: five minutes at 95°C, five seconds at 95°C, 20 seconds at 59°C and 10 seconds at 72°C. The relative expression levels for each sample were calculated based on the findings of three technical replicates, and the amplification products were validated using a melting curve analysis.

Ultrasound Examinations

B-mode ultrasound studies of the left and right carotid artery were performed using a 7.5 to 10.0 MHz linear array transducer (P700SE; Phillips Medi-

Table 1. Characteristics of the ischemic stroke (IS) and control groups

Variables	IS (n=342)	control (n=296)	p value
Mean age (years)	70.30 ± 10.75	70.41 ± 8.57	0.893
Male/female	228/114	188/108	0.404
Smokers, n (%)	173 (50.58%)	54 (18.24%)	<0.001
Hypertension, n (%)	192 (54.86%)	65 (21.96%)	<0.001
Diabetes, n (%)	70 (20%)	33 (11.15%)	0.001
Blood glucose (mmol/L)	5.94 ± 2.54	5.46 ± 1.64	0.007
Total cholesterol (mg/dL)	5.20 ± 1.04	4.78 ± 1.09	<0.001
Triglycerides (mmol/L)	1.57 ± 0.97	1.58 ± 1.43	0.893
HDL (mmol/L)	1.27 ± 0.48	1.58 ± 0.40	<0.001
LDL (mmol/L)	2.78 ± 0.90	2.91 ± 0.93	0.084

Continuous data are expressed as the mean ± SD

cal System). The optimized images of the left and right carotid artery intima-media thickness (IMT) were selected and frozen at the end of diastole. The IMT of the CCA was measured with the automated ultrasonic software Calcs program (to trace the lumen-intima and media-adventitia interfaces) in the near and far walls on both the left and right sides in an area free of atherosclerotic plaques. The value of the IMT of the CCA in the far wall of each side was used for the analysis. The near and far walls of the CCA, the carotid bifurcations and the origins of the external and internal carotid arteries of both sides were scanned longitudinally and transversely for the presence of atherosclerotic plaques, which were defined by the appearance of the largest focal lesion (that exhibiting protrusion into the lumen of 1.5 mm in thickness) in shape and texture on the longitudinal images. Then, the plaques were confirmed on cross-sectional views of the lumen. The maximum thickness of each plaque was measured with ultrasonic calipers and recorded on the left or right side.

Statistical Analysis

All statistical tests were performed using the SPSS 19.0 software program (IBM, Armonk, NY, USA). The Hardy-Weinberg equilibrium of the SNPs was examined using the HWE software program. The clinical data are expressed as the mean ± standard deviation (SD) for continuous variables and the median or percentage for quantitative variables; the chi-squared test and Student's *t*-test were used to compare variables between the two groups. The allele and genotype frequency differences between the patients and healthy controls were assessed using the χ^2 test or Fisher's exact test. The odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the correlation between the *ADAM17* genotype and IS. The haplotype analyses were conducted using the Haploview

software program, version 3.2.0. The associations between the gene polymorphisms and CIMT were analyzed using a multiple linear regression model (SAS, version 6.12; SAS Institute Inc., Cary, NC, USA). *P* values of less than 0.05 were considered to be statistically significant.

Results

Baseline Characteristics

The clinical characteristics of the 638 participants (342 IS patients and 296 control subjects) are presented in **Table 1**. Diabetes, hypertension and smoking were significantly more common in the IS group than in the control group. There were no significant differences in age or sex between the IS patients and controls. The mean ages of the IS and control patients were 70.30 years (±10.75 years) and 70.41 years (±8.57 years), respectively. In the IS patients, the total cholesterol and blood glucose levels tended to be higher than those observed in the controls, while the high-density lipoprotein (HDL) levels were lower at admission. The triglyceride and low-density lipoprotein (LDL) levels in the IS patients were not significantly different from those noted in the healthy control subjects.

ADAM17 Gene Polymorphisms and the Risk of IS

The genotype and allele frequencies of the *ADAM17* polymorphisms are shown in **Table 2**. No deviation from the Hardy-Weinberg equilibrium was observed for the polymorphisms examined with respect to the genotype distributions of the IS patients and controls (data not shown). Because the homozygous minor allele carriers were limited in number, we analyzed three *ADAM17* SNPs (rs11684747 A>G, rs11689958 G>A and rs55790676 G>T) using three

Table 2. Frequencies of the *ADAM17* genotypes and alleles in the IS patients and control subjects

Genotypes	Cases <i>n</i> =342 (%)	Controls <i>n</i> =296 (%)	AOR (95%CI)	<i>p</i> value
rs11684747				
A/A	327 (95.61)	283 (95.61)		
G/A + G/G ^a	15 (4.39)	13 (4.39)		
Dominant model AA/GA vs. GG	341 (99.71)	296 (1)	0.997, 0.798-1.246	0.979
Recessive model AA vs. GA/GG	15 (4.39)	13 (4.39)	0.999, 0.467-2.134	0.997
A allele	668 (0.977)	579 (0.978)	1.000 (reference)	
G allele	16 (0.023)	13 (0.022)	0.94, 0.45-1.97	0.864
rs11689958				
G/G	327 (95.61)	283 (95.61)		
G/A + A/A ^a	15 (4.39)	13 (4.39)		
Dominant model GG/GA vs. AA	341 (99.71)	296 (1)	0.997, 0.798-1.246	0.979
Recessive model GG vs. GA/AA	15 (4.39)	13 (4.39)	0.999, 0.467-2.134	0.997
G allele	668 (0.977)	579 (0.978)	1.000 (reference)	
A allele	16 (0.023)	13 (0.022)	0.94, 0.45-1.97	0.864
rs12692386				
A/A	203 (59.36)	172 (58.11)		
G/A	123 (35.96)	114 (38.51)		
G/G	16 (4.68)	10 (3.38)		0.613
Dominant model AA/GA vs. GG	326 (95.32)	286 (96.62)	0.71, 0.32-1.59	0.408
Recessive model AA vs. GA/GG	139 (40.64)	124 (41.22)	1.05, 0.77-1.44	0.827
A allele	529 (0.773)	458 (0.774)	1.000 (reference)	
G allele	155 (0.227)	134 (0.226)	1, 0.77-1.30	0.991
rs55790676				
G/G	327 (95.61)	283 (95.61)		
G/T + T/T ^a	15 (4.10)	13 (4.39)		
Dominant model GG/GT vs. TT	341 (99.71)	296 (1)	0.997, 0.798-1.246	0.979
Recessive model GG vs. GT/TT	15 (4.39)	13 (4.39)	0.999, 0.467-2.134	0.997
G allele	668 (0.977)	579 (0.978)	1.000 (reference)	
T allele	16 (0.023)	13 (0.022)	0.94, 0.45-1.97	0.864
rs1524668				
A/A	260 (76.02)	230 (0.88)		
A/C	79 (23.10)	65 (0.88)		
C/C	3 (0.88)	1 (0.34)		0.642
Dominant model AA/AC vs. CC	339 (99.12)	295 (99.66)	0.38, 0.04-3.7	0.616
Recessive model CC vs. AC/AA	82 (23.98)	66 (22.30)	0.91, 0.63-1.32	0.389
A allele	599 (0.876)	525 (0.887)	1.000 (reference)	
C allele	85 (0.124)	67 (0.113)	0.90, 0.64-1.26	0.542

^a*p* value of the difference in genotype between the cases and controls^b*p* value of the difference in alleles between the cases and controls

AOR: adjusted odds ratio

*Three genotype categories (AA vs. AG + GG, GG + GA vs. AA and GG vs. GT + TT) were used to analyze the three *ADAM17* SNPs (rs11684747 A > G, rs11689958 G > A and rs55790676 G > T, respectively) because the homozygous minor allele carriers were few in number.

genotype categories (AA vs. AG + GG, GG + GA vs. AA and GG vs. GT + TT). The overall genotype and allele distributions of the *ADAM17* polymorphisms did not differ significantly between the IS patients and the healthy control subjects ($p > 0.05$).

We further performed a haplotype-based association analysis of the *ADAM17* polymorphisms using the Haploview software program. However, no significant associations were observed between the haplotypes and IS (**Table 3**).

Table 3. Frequencies of the *ADAM17* haplotypes in the IS patients and healthy controls

Haplotypes	Cases <i>n</i> = 684 (%)	Controls <i>n</i> = 592 (%)	χ^2	<i>p</i> value
GAAAG	529.8 (77.5%)	457.9 (77.3%)	0.003	0.959
GGAAG	69.2 (10.1%)	68.1 (11.5%)	0.646	0.421
GGACG	68.8 (10.1%)	50.9 (8.6%)	0.809	0.369

Table 4. Quantitative traits stratified according to the *ADAM17* genotype in the IS patients and control subjects

Characteristics	Case group genotype		Control group genotype		<i>p</i> value	
rs11684747	AA	GA + GG	AA	GA + GG	P _{AA}	P _{GA+GG}
Blood glucose (mmol/L)	5.95 ± 2.57	5.86 ± 1.74	5.50 ± 1.67	4.81 ± 0.51	0.013	0.039
Total cholesterol (mg/dL)	5.21 ± 1.05	5.02 ± 0.68	4.76 ± 1.08	5.31 ± 1.10	< 0.001	0.414
Triglycerides (mmol/L)	1.57 ± 0.99	1.54 ± 0.46	1.59 ± 1.41	1.47 ± 1.81	0.868	0.889
HDL (mmol/L)	1.26 ± 0.48	1.45 ± 0.50	1.58 ± 0.40	1.62 ± 0.47	< 0.001	0.344
LDL (mmol/L)	2.77 ± 0.90	3.09 ± 0.86	2.92 ± 0.94	2.80 ± 0.57	0.050	0.29
rs11689958	GG	GA + AA	GG	GA + AA	P _{GG}	P _{GA+AA}
Blood glucose (mmol/L)	5.95 ± 2.57	5.86 ± 1.74	5.50 ± 1.67	4.81 ± 0.51	0.013	0.039
Total cholesterol (mg/dL)	5.21 ± 1.05	5.02 ± 0.68	4.76 ± 1.08	5.31 ± 1.10	< 0.001	0.414
Triglycerides (mmol/L)	1.57 ± 0.99	1.54 ± 0.46	1.59 ± 1.41	1.47 ± 1.81	0.868	0.889
HDL (mmol/L)	1.26 ± 0.48	1.45 ± 0.50	1.58 ± 0.40	1.62 ± 0.47	< 0.001	0.344
LDL (mmol/L)	2.77 ± 0.90	3.03 ± 0.86	2.92 ± 0.94	2.80 ± 0.57	0.050	0.290
rs12692386	AA	GA + GG	AA	GA + GG	P _{AA}	P _{GA+GG}
Blood glucose (mmol/L)	5.88 ± 2.46	5.99 ± 2.64	5.40 ± 1.56	5.34 ± 1.73	0.081	0.037
Total cholesterol (mg/dL)	5.23 ± 0.98	5.18 ± 1.08	4.78 ± 1.10	4.78 ± 1.07	< 0.001	0.003
Triglycerides (mmol/L)	1.55 ± 1.04	1.60 ± 0.87	1.64 ± 1.65	1.49 ± 1.02	0.529	0.384
HDL (mmol/L)	1.28 ± 0.54	1.26 ± 0.39	1.59 ± 0.40	1.58 ± 0.40	< 0.001	< 0.001
LDL (mmol/L)	2.80 ± 0.92	2.76 ± 0.87	2.91 ± 0.88	2.89 ± 1.00	0.238	0.257
rs55790676	GG	GT + TT	GG	GT + TT	P _{GG}	P _{GT+TT}
Blood glucose (mmol/L)	5.93 ± 2.57	5.77 ± 1.77	5.49 ± 1.66	4.81 ± 0.51	0.013	0.039
Total cholesterol (mg/dL)	5.21 ± 1.05	5.02 ± 0.68	4.76 ± 1.08	5.31 ± 1.10	< 0.001	0.414
Triglycerides (mmol/L)	1.58 ± 0.99	1.53 ± 0.47	1.59 ± 1.40	1.17 ± 1.81	0.868	0.889
HDL (mmol/L)	1.26 ± 0.48	1.46 ± 0.49	1.58 ± 0.40	1.62 ± 0.47	< 0.001	0.344
LDL (mmol/L)	2.77 ± 0.50	2.99 ± 0.84	2.91 ± 0.95	2.80 ± 0.57	0.050	0.29
rs1524668	AA	AC + CC	AA	AC + CC	P _{AA}	P _{AC+CC}
Blood glucose (mmol/L)	6.0 ± 2.63	5.75 ± 2.23	5.53 ± 1.72	5.23 ± 1.26	0.022	0.094
Total cholesterol (mg/dL)	5.21 ± 1.01	5.16 ± 1.11	4.79 ± 1.11	4.74 ± 1.00	< 0.001	0.018
Triglycerides (mmol/L)	1.59 ± 1.02	1.53 ± 0.80	1.64 ± 1.52	1.38 ± 1.00	0.669	0.377
HDL (mmol/L)	1.27 ± 0.51	1.28 ± 0.38	1.59 ± 0.40	1.57 ± 0.41	< 0.001	< 0.001
LDL (mmol/L)	2.80 ± 0.91	2.74 ± 0.86	2.90 ± 0.90	2.93 ± 1.07	0.234	0.238

The data are expressed as the mean ± SD.

P_{AA}: *p* value of the difference in the AA genotype between the cases and controls; P_{GA}: *p* value of the difference in the GA genotype between the cases and controls; P_{GG}: *p* value of the difference in the GG genotype between the cases and controls; P_{GT}: *p* value of the difference in the GT genotype between the cases and controls; P_{TT}: *p* value of the difference in the TT genotype between the cases and controls; P_{CC}: *p* value of the difference in the CC genotype between the cases and controls; P_{AC}: *p* value of the difference in the AC genotype between the cases and controls

Associations between the ADAM17 Gene Polymorphisms and Demographic Characteristics

The associations between the *ADAM17* gene polymorphisms and demographic characteristics are

shown in **Supplementary Tables 1-5**. No significant differences in the genotype or allele frequencies were detected between the IS patients and healthy control subjects in terms of age, sex, hypertension, smoking or

Table 5. Stratification analysis of the relationships between the *ADAM17* polymorphisms and stroke

Genotypes	Controls	OCSF classification				TOAST classification	
		TACI (%)	PACI (%)	POCI (%)	LACI (%)	LAA (%)	SAA (%)
rs11684747							
AA	283 (95.6)	34 (97.1)	145 (92.4)	50 (100)	98 (98)	161 (95.3)	166 (96)
GA/GG	13 (4.4)	1 (2.9)	12 (7.6)	0	2 (2)	8 (4.7)	7 (4)
<i>p</i> value	Ref	0.67	0.149	0.131	0.279	0.864	0.858
rs11689958							
GG	283 (95.6)	34 (97.1)	145 (92.4)	50 (100)	98 (98)	161 (95.3)	166 (96)
GA/AA	13 (4.4)	1 (2.9)	12 (7.6)	0	2 (2)	8 (4.7)	7 (4)
<i>p</i> value	Ref	0.67	0.149	0.131	0.279	0.864	0.858
rs12692386							
AA	172 (58.1)	24 (68.6)	90 (57.3)	33 (66)	56 (56)	97 (57.4)	106 (61.3)
GA/GG	124 (41.9)	11 (31.4)	67 (42.7)	17 (34)	44 (44)	72 (42.6)	67 (38.7)
<i>p</i> value	Ref	0.234	0.872	0.294	0.712	0.881	0.501
rs55790676							
GG	283 (95.6)	34 (97.1)	145 (92.4)	50 (100)	98 (98)	161 (95.3)	166 (96)
GT/TT	13 (4.4)	1 (2.9)	12 (7.6)	0	2 (2)	8 (4.7)	7 (4)
<i>p</i> value	Ref	0.67	0.149	0.131	0.279	0.864	0.858
rs1524668							
AA	230 (77.7)	30 (85.7)	108 (68.8)	42 (84)	80 (80)	126 (74.6)	134 (77.5)
AC/CC	66 (22.3)	5 (14.3)	49 (31.2)	8 (16)	20 (20)	43 (25.4)	39 (22.5)
<i>p</i> value	Ref	0.275	0.038	0.315	0.63	0.441	0.951

All values are adjusted for age, sex, hypertension, diabetes, hypercholesterolemia and smoking habits according to a multivariate regression analysis. TACI=total anterior circulation infarct; PACI=partial anterior circulation infarct; LACI=lacunar infarct; POCI=posterior circulation infarct; LAA=large-artery atherosclerosis; SAA=small-artery atherosclerosis

diabetes ($p > 0.05$).

We next examined the associations between *ADAM17* polymorphisms and anthropometric measurements, including the blood glucose, total cholesterol, triglyceride, HDL and LDL levels. The results are shown in **Table 4**. For the rs11684747, rs11689958 and rs55790676 *ADAM17* polymorphisms, the variation in these biomarkers was similar. The blood glucose levels were elevated in the IS group with both the major common allele and minor mutated allele compared with that observed in the control group ($p < 0.05$). The carriers of the major AA or GG allele displayed higher total cholesterol but lower HDL concentrations than the patients with the mutated GA + GG, GA + AA or GA + GG alleles. For the rs12692386 and rs1524668 polymorphisms of *ADAM17*, higher total cholesterol concentrations ($p < 0.05$) and lower HDL concentrations ($p < 0.001$) were observed in the IS patients than in the control subjects. However, higher blood glucose concentrations were detected in patients with the major AA allele of rs1524668 ($p = 0.022$) and those with the mutated GA + GG allele of rs12692386 ($p = 0.037$).

Associations between the *ADAM17* Gene Polymorphisms and Stroke Subtype

To explore whether the *ADAM17* polymorphisms are confined to a specific subtype or related to generalized risk, we further separated the IS patient groups into stroke subgroups according to the OCSF¹⁵⁾ and TOAST¹²⁾ classifications. As shown in **Table 5**, when the population was stratified according to the OCSF classification system (total anterior circulation infarct, TACI; partial anterior circulation infarct, PACI; lacunar infarct, LACI; and posterior circulation infarct, POCI), the carriers of the rs1524668 C *ADAM17* allele mutation appeared to have a higher risk of stroke of the PACI subtype compared with the controls ($p = 0.038$). However, when the population was stratified according to the TOAST classification system (large artery atherosclerosis, LAA; small-artery atherosclerosis, SAA), no statistical associations were observed with respect to the *ADAM17* polymorphisms of the two major stroke subtypes in the healthy controls.

Fig. 1. (A) Mean relative ADAM17 mRNA values $\pm$ SD in the PBMCs obtained from the IS patients (IS, $n=100$) and healthy control subjects (controls, $n=100$). The blank box and black box represent the relative expression levels of ADAM17 in the controls and patients with IS, respectively. $P=0.77$ when comparing the relative ADAM17 mRNA levels between the IS patients and controls. (B) Mean relative ADAM17 mRNA values $\pm$ SD in the PBMCs obtained from the IS patients (IS, $n=100$) and healthy control subjects (controls, $n=100$) stratified according to the diabetic status. The blank box and black box represent the relative expression levels of ADAM17 in the diabetic and non-diabetic individuals, respectively. $p>0.05$

Effects of ADAM17 Gene Polymorphisms on the ADAM17 Expression

A comparative determination was performed regarding the ADAM17 mRNA expression levels in the PBMCs of 50 IS patients and 50 healthy controls. No significant differences were observed in the mean ADAM17 mRNA expression levels of the IS patients and controls (**Fig. 1A**) ($p=0.56$). We also determined the ADAM17 mRNA expression levels in the patients and controls stratified according to the diabetic status. However, no differences were found in the ADAM17 mRNA expression between the diabetic and non-diabetic individuals (**Fig. 1B**) ($p>0.05$), which supports our previous findings that there are no associations between ADAM17 promoter polymorphisms and the risk of developing IS, even when the diabetes status is taken into consideration.

In addition, we assessed whether the mean ADAM17 mRNA levels and the *ADAM17* genotype were associated in the IS patients. The results are presented in **Fig. 2**. A significant increase in the ADAM17 mRNA expression was observed in IS patients who carried the mutated rs12692386 G allele ($p=0.042$). A similar trend, although not significant, was observed in the patients carrying the correspondingly mutated alleles of the other four *ADAM17* polymorphisms (rs11684747, rs11689958, rs55790676 and rs1524668).

Effects of ADAM17 Gene Polymorphisms on Carotid Atherosclerosis

The associations of the *ADAM17* gene polymorphisms with CIMT and IS were explored, and the results are presented in **Fig. 3**. The mean CIMT of the IS patients with minor variant genotypes (rs11684747 GA+AA, rs11689958 GA+GG, rs55790676 GT+TT, rs1524668 GA+GG and rs12692386 AC+CC) was lower, although not significantly, than that observed in those with the major genotypes (rs11684747 GG, rs11689958 AA, rs55790676 GG, rs1524668 AA and rs12692386 AA) ($p>0.05$).

Discussion

In this hospital-based case-control study, we show, for the first time, that the five SNPs in the *ADAM17* promoter region are not major contributors to the pathogenesis of IS. However, further stratification revealed that the C allele of the *ADAM17* rs1524668 polymorphism is associated with a higher risk of IS in the PACI subtype. Additionally, the *ADAM17* rs12692386 A>G polymorphism in the promoter region may affect the expression of ADAM17.

Accumulating evidence suggests the potential impact of ADAM family members on the pathogenic mechanisms leading to IS^{4, 16}. By functioning as

Fig. 2. Mean ADAM17 mRNA values $\pm$ SD in the PBMCs isolated from the IS patients ($n=100$) stratified according to the presence of the mutated allele ($p=0.042$ for rs12692386).

Fig. 3. Mean IMT values $\pm$ SD (cm) in the IS patients ($n=264$) stratified according to the presence of the mutated allele, $p>0.05$

molecular scissors, ADAM proteases play a role in various biological functions, including cell migration, adhesion, angiogenesis, cell surface marker activation and proteolysis. ADAM17, also known as the TNF- α -converting enzyme (TACE), produces the precursor of TNF- α via a process called shedding¹⁷. This enzyme cleaves a wide diversity of substrates, includ-

ing TNFR1, TNFR2, IL-6R, L-selectin, CD30, APP, collagen XVII and EGFR, some of which have been suggested to play an essential role in atherosclerotic plaque progression¹⁸. ADAM17 has been shown to regulate the growth factor and hormone signaling of several epithelial growth factor (EGF) family members. Therefore, the increased expression of ADAM17

may further facilitate the proliferation and invasiveness of inflammatory cells, such as leukocytes, in atherosclerotic lesions and therefore contribute to plaque progression³). The observations that ADAM17 is primarily localized in macrophages and that its expression is significantly increased in plaques have confirmed its involvement in the pathogenesis of IS. Despite these advances, the *ADAM17* alleles contributing to IS remain undiscovered.

A previous study indicated that two *ADAM17* polymorphisms, C-154A and Ser747Leu, slightly influence the TNF plasma level and the risk of cardiovascular death, respectively¹⁰). Furthermore, Junyent *et al.* found that *ADAM17* polymorphisms (rs11684747 and rs10495563) are linked with obesity risk and that the *ADAM17* rs10495563 SNP is associated with a low intake of (n-6) dietary polyunsaturated fatty acids¹¹). Meanwhile, Wang *et al.* recently reported that there are no associations between *ADAM17* polymorphisms (-1672C/T, -1638T/G, -1437T/C, -1333C/T, -172T/C and -154C/A) and sporadic AD¹⁴).

As an increased ADAM17 expression has previously been observed in unstable plaques, especially those involving macrophages^{16, 19}), we examined the ADAM17 mRNA levels in peripheral blood mononuclear cells and found them to be increased 1.1-fold in the IS patients compared to the controls, although the difference was not significant. However, the mRNA levels in PBMCs may not reflect the expression observed in tissues, and the polymorphism may have a strong effect on plaques¹⁰). The lack of significance of the ADAM17 mRNA expression levels in this case-control analysis is likely due to the comparatively small number of samples examined. We observed that IS patients carrying a mutated rs12692386 G *ADAM17* allele have a higher expression of ADAM17 than those with a major A allele. This study is the first report to show that the rs12692386 polymorphism of *ADAM17* affects the ADAM17 mRNA expression, although Dixon *et al.*²⁰) previously demonstrated that the ADAM17_i62781 G>T polymorphism regulates the *ADAM17* gene expression. The mechanism by which the rs12692386 *ADAM17* polymorphism contributes to elevation of the ADAM17 expression is unknown. Given that five of the analyzed SNPs map to promoter regions, the presence of transcriptional enhancers and other regulatory elements observed in the promoter regions may play various regulatory roles in this phenomenon. In this regard, with respect to the rs12692386 A>G polymorphism, a computational analysis using MAPPER indicated a potential allele-specific binding site for the upstream stimulatory factor (USF) transcription factor. It is conceivable that

the rs12692386 A>G polymorphism of *ADAM17* affects its binding to USF, thereby influencing the regulation of the gene expression.

Stroke is a heterogeneous phenotype, and therefore an analysis of the incidence of stroke may overlook genetic effects that are restricted to a certain stroke subtypes. OCSF is a clinical classification that provides information regarding the extent of the lesion²¹). Bamford *et al.* reported that, in their study, the patients in the PACI group were much more likely to have episodes of early recurrent stroke than the patients in other groups¹³). Our study demonstrated that the *ADAM17* polymorphisms exhibit a different distribution according to the OCSF subtype; accordingly, the C allele of the *ADAM17* rs1524668 polymorphism was found to be associated with a higher risk of IS in the PACI subtype. Therefore, to some extent, patients with a mutant rs1524668 C allele of *ADAM17* may have a higher risk of a poor outcome. However, the association between the rs1524668 polymorphism of *ADAM17* and the PACI stroke subtype was not large ($p=0.038$), which may be due to the comparatively small sample size. Therefore, it is necessary to confirm this observation in a larger sample size in future studies.

The carotid artery intima-media wall thickness (IMT) is a useful measurement of atherosclerosis. A previous study showed that subjects with the MMP3 genotype 6A have a greater mean carotid IMT than those with other MMP3 genotypes combined²²). The progression of atherosclerosis in carotid artery lesions likely results from the excessive degradation of the extracellular matrix components of the vessel wall, particularly elastic and collagen fibers^{23, 24}). ADAMs, proteins that are evolutionarily related to MMPs and play significant roles in atherosclerotic carotid plaque progression, may be involved in early vascular remodeling, thus affecting CIMT thickening. Our previous study also indicated an association between the rs514049 A ADAM10 allele and a higher CIMT than the mutated C allele among patients with atherosclerotic cerebral infarction²⁵). In the present study, no associations were found between *ADAM17* polymorphisms and CIMT in the IS patients. Our findings provide little support for *ADAM17* polymorphisms being direct risk factors for IMT. However, the average CIMT tended to be lower in the individuals with the minor allele across the *ADAM17* SNPs, which is in agreement with the findings of our previous study showing the mean CIMT in patients with ACI carrying the minor C allele of rs514049 AA genotype to be lower than that observed in patients carrying a major C allele. Nevertheless, the exact mechanism remains

under investigation. IMT provides a measurement of early atherosclerosis and remodeling; however, little information is available regarding the processes involved in later stages of atherosclerosis and plaque rupture. Notably, although they share many risk factors, IMT, plaque and cerebral infarction are distinct phenotypes in which multiple factors are involved. Hence, the effects of *ADAM17* polymorphisms on the IMT should be further investigated with larger sample sizes.

Inflammation and dyslipidemia are important risk factors involved in the onset and progression of atherosclerosis. A significantly positive correlation between TNF- α and lipoprotein is observed in patients with coronary artery disease²⁶. Meanwhile, patients with obesity and dyslipidemia exhibit increased TNF- α levels, and the inactivation of TNF- α may affect lipid metabolism, including that of cholesterol and lipoproteins²⁷. In the current study, the total cholesterol levels were significantly higher and the HDL levels were significantly lower in the IS patients carrying the major allele of rs11684747 (AA), rs11689958 (GG) or rs55790676 (GG) *ADAM17* polymorphisms than in the control group, suggesting that these polymorphisms may be specifically linked to total cholesterol and HDL variation. TNF- α is necessary for the maintenance of glucose homeostasis²⁸, and the administration of prolonged TNF- α antagonism therapy improves the fasting glucose level²⁹. In the present study, the blood glucose levels were found to be significantly elevated in the patients carrying the mutated GA + GG allele with rs12692386, whereas these levels were decreased in the IS patients with the major AA allele of rs1524668. In addition, the expression of *ADAM17* mRNA was elevated in the IS patients carrying the mutated allele of the five *ADAM17* polymorphisms (as shown in **Fig. 2**); however, only the rs12692386 polymorphism reached a significant level. Therefore, it is conceivable that *ADAM17* polymorphisms influence the release of TNF- α by altering the *ADAM17* expression, which affects the variation in the total cholesterol, HDL and blood glucose levels.

TNF- α is a target of various anti-inflammatory therapies; therefore, the inhibition of *ADAM17* may be a viable therapeutic strategy in the treatment of inflammation and/or inflammation-associated diseases³⁰. In humans with metabolic disorders, such as obesity and type 2 diabetes, the co-occurrence of lipotoxicity and glucotoxicity has been found to increase the *ADAM17* activity by decreasing the tissue inhibitor of metalloproteinase 3 (TIMP3) expression, while an increased *ADAM17* activity has been reported to correlate with insulin resistance³¹. Therefore, *ADAM17*

blockade may emerge as a potential target of therapy for metabolic inflammation. TIMP3, a key endogenous inhibitor involved in the regulation of the activity of matrix metalloproteinases (MMPs) and ADAMs, is the only known physiological inhibitor of *ADAM17*³². The overexpression of TIMP3 inhibits metabolic inflammation and related metabolic disorders, such as those involving insulin resistance, glucose intolerance and nonalcoholic steatohepatitis³³. Conversely, a decreased TIMP3 expression leads to an increased expression of *ADAM17* in diabetic patients and is correlated with insulin resistance and an increased IMT thickness. Furthermore, the overexpression of TIMP3 decreases the inflammatory content in LDLR-/- mice³⁴. Other inhibitors of the *ADAM17* blockade target iRhom2, which belongs to a class of polytopic endoplasmic reticulum proteins and has been identified to be an essential factor for the activity and trafficking of *ADAM17*^{35, 36}. Additionally, deacetylase Sirtuin 1 (SirT1) overexpression has been shown to be capable of increasing the TIMP3 activity, leading to a decrease in the *ADAM17* expression in the context of diabetes and atherosclerotic diseases³⁷. In the present study, we determined the *ADAM17* mRNA expression levels in both the patients and controls stratified according to a diabetic or non-diabetic status. However, we found no differences in the *ADAM17* mRNA expression between the diabetic and non-diabetic individuals, which supports our previous findings that there are no associations between *ADAM17* promoter polymorphisms and the risk of developing IS, even when the diabetes status is taken into consideration.

Despite this evidence, our data should be interpreted with caution. For example, the case-control design of this study may weaken or distort any true relationship between *ADAM17* and IS. Therefore, large prospective studies with a long period of follow-up are required. Other clinical characteristics of the study group, such as the rates of hypertension, diabetes or hypercholesterolemia, may have masked the associations between the *ADAM17* polymorphisms and IS. Furthermore, other functional polymorphisms may also influence the *ADAM17* expression, and their combined effects must be studied in order to improve the ability to predict the occurrence, severity and outcomes of IS.

In conclusion, polymorphisms in the *ADAM17* promoter were determined to be unrelated to the risk of IS in a Chinese population. However, the rs1524668 *ADAM17* polymorphism was found to be associated with a higher risk of the PACI type of stroke, and the rs12692386 A>G *ADAM17* polymorphism may influence the *ADAM17* expression.

Therefore, the rs1524668 ADAM17 polymorphism is likely to be one a key determinant of an increased occurrence of the PACI stroke subtype, while elevation of the ADAM17 mRNA expression confirms the role of ADAM17 in the pathophysiology of IS, with potentially important therapeutic implications. However, this study is only retrospective and involves multiple comparisons. Therefore, prospective studies with a comparatively larger sample size are required for independent validation.

Acknowledgements

This work was supported by funding from the National Nature Science Foundation of China (grant numbers 31171219, 81271213, 81070878, 81271214, 81300929 and 81261120404), the Natural Science Foundation of Guangdong Province, China (No. S2012010008222) and the Science and Technology Innovation Fund of Guangdong Medical College (No. STIF 201101).

Conflict of Interest Statement

The authors have no actual or potential conflicts of interest related to this manuscript. Appropriate approval was obtained, and appropriate procedures were followed concerning human subjects.

References

- Lo AC, Guarino PD, Richards LG, Haselkorn JK, Wittenberg GF, Federman DG, Ringer RJ, Wagner TH, Krebs HI, Volpe BT: Robot-assisted therapy for long-term upper-limb impairment after stroke. *N Engl J Med*, 2010; 362: 1772-1783
- Pelisek J, Eckstein H-H, Zerneck A: Pathophysiological mechanisms of carotid plaque vulnerability: impact on ischemic stroke. *Arch Immunol Ther Exp (Warsz)*, 2012; 60: 431-442
- Saftig P, Reiss K: The "A Disintegrin And Metalloproteases" ADAM10 and ADAM17: Novel drug targets with therapeutic potential? *Eur J Cell Biol*, 2011; 90: 527-535
- van der Vorst EP, Keijbeck AA, de Winther MP, Donners MM: A disintegrin and metalloproteases: molecular scissors in angiogenesis, inflammation and atherosclerosis. *Atherosclerosis*, 2012; 224: 302-308
- Dreymueller D, Pruessmeyer J, Groth E, Ludwig A: The role of ADAM-mediated shedding in vascular biology. *Eur J Cell Biol*, 2012; 91: 472-485
- Oksala N, Levula M, Airla N, Peltto-Huikko M, Ortiz RM, Jarvinen O, Salenius JP, Ozsait B, Komurcu-Bayrak E, Erginel-Unaltuna N, Huovila AP, Kytomaki L, Soini JT, Kahonen M, Karhunen PJ, Laaksonen R, Lehtimäki T: ADAM-9, ADAM-15, and ADAM-17 are upregulated in macrophages in advanced human atherosclerotic plaques in aorta and carotid and femoral arteries--Tampere vascular study. *Ann Med*, 2009; 41: 279-290
- Satoh M, Ishikawa Y, Itoh T, Minami Y, Takahashi Y, Nakamura M: The expression of TNF- α converting enzyme at the site of ruptured plaques in patients with acute myocardial infarction. *Eur J Clin Invest*, 2008; 38: 97-105
- Canault M, Leroyer AS, Peiretti F, Leseche G, Tedgui A, Bonardo B, Alessi MC, Boulanger CM, Nalbone G: Microparticles of human atherosclerotic plaques enhance the shedding of the tumor necrosis factor- α converting enzyme/ADAM17 substrates, tumor necrosis factor and tumor necrosis factor receptor-1. *Am J Pathol*, 2007; 171: 1713-1723
- Holdt LM, Thiery J, Breslow JL, Teupser D: Increased ADAM17 mRNA expression and activity is associated with atherosclerosis resistance in LDL-receptor deficient mice. *Arterioscler Thromb Vasc Biol*, 2008; 28: 1097-1103
- Morange PE, Tregouet DA, Godefroy T, Saut N, Bickel C, Rupprecht HJ, Lackner K, Barbaux S, Poirier O, Peiretti F, Nalbone G, Juhan-Vague I, Blankenberg S, Tiret L: Polymorphisms of the tumor necrosis factor- α (TNF) and the TNF- α converting enzyme (TACE/ADAM17) genes in relation to cardiovascular mortality: the AtheroGene study. *J Mol Med (Berl)*, 2008; 86: 1153-1161
- Junyent M, Parnell LD, Lai CQ, Arnett DK, Tsai MY, Kabagambe EK, Straka RJ, Province M, An P, Smith CE, Lee YC, Borecki I, Ordovas JM: ADAM17_i33708A>G polymorphism interacts with dietary n-6 polyunsaturated fatty acids to modulate obesity risk in the Genetics of Lipid Lowering Drugs and Diet Network study. *Nutr Metab Cardiovasc Dis*, 2010; 20: 698-705
- Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE, 3rd: Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke*, 1993; 24: 35-41
- Bamford J, Sandercock P, Dennis M, Burn J, Warlow C: Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet*, 1991; 337: 1521-1526
- Wang M, Li Y, Lu Y, Zuo X, Wang F, Zhang Z, Jia J: The relationship between ADAM17 promoter polymorphisms and sporadic Alzheimer's disease in a Northern Chinese Han population. *J Clin Neurosci*, 2010; 17: 1276-1279
- Pitcock SJ, Meldrum D, Hardiman O, Thornton J, Brennan P, Moroney JT: The Oxfordshire Community Stroke Project classification: correlation with imaging, associated complications, and prediction of outcome in acute ischemic stroke. *J Stroke Cerebrovasc Dis*, 2003; 12: 1-7
- Dreymueller D, Pruessmeyer J, Groth E, Ludwig A: The role of ADAM-mediated shedding in vascular biology. *Eur J Cell Biol*, 2012; 91: 472-485
- Clackson-Welsh L: ADAM-mediated shedding, a new flavor in angiogenesis regulation. *Arterioscler Thromb Vasc Biol*, 2010; 30: 2087-2088
- Edwards DR, Handsley MM, Pennington CJ: The ADAM metalloproteinases. *Mol Aspects Med*, 2008; 29: 258-289

- 19) Pelisek J, Pongratz J, Deutsch L, Reeps C, Stadlbauer T, Eckstein HH: Expression and cellular localization of metalloproteases ADAMs in high graded carotid artery lesions. *Scand J Clin Lab Invest*, 2012; 72: 648-656
- 20) Dixon AL, Liang L, Moffatt MF, Chen W, Heath S, Wong KC, Taylor J, Burnett E, Gut I, Farrall M, Lathrop GM, Abecasis GR, Cookson WO: A genome-wide association study of global gene expression. *Nat Genet*, 2007; 39: 1202-1207
- 21) Luo Y, Wang Z, Li J, Xu Y: Serum CRP concentrations and severity of ischemic stroke subtypes. *Can J Neurol Sci*, 2012; 39: 69-73
- 22) Armstrong C, Abilleira S, Sitzler M, Markus HS, Bevan S: Polymorphisms in MMP family and TIMP genes and carotid artery intima-media thickness. *Stroke*, 2007; 38: 2895-2899
- 23) Dollery CM, Libby P: Atherosclerosis and proteinase activation. *Cardiovasc Res*, 2006; 69: 625-635
- 24) Back M, Ketelhuth DF, Agewall S: Matrix metalloproteinases in atherothrombosis. *Prog Cardiovasc Dis*, 2010; 52: 410-428
- 25) Li Y, Liao F, Yin XJ, Cui LL, Ma GD, Nong XX, Zhou HH, Chen YF, Zhao B, Li KS: An Association Study on ADAM10 Promoter Polymorphisms and Atherosclerotic Cerebral Infarction in a Chinese Population. *CNS Neurosci Ther*, 2013;
- 26) Goswami B, Rajappa M, Singh B, Ray P, Kumar S, Malika V: Inflammation and dyslipidaemia: a possible interplay between established risk factors in North Indian males with coronary artery disease. *Cardiovasc J Afr*, 2010; 21: 103-108
- 27) MM RA: [Tumor necrosis factor- α , insulin resistance, the lipoprotein metabolism and obesity in humans]. *Nutricion hospitalaria: organo oficial de la Sociedad Espanola de Nutricion Parenteral y Enteral*, 2012; 27: 1751-1757
- 28) Lacerda L, Opie LH, Lecour S: Influence of tumour necrosis factor alpha on the outcome of ischaemic post-conditioning in the presence of obesity and diabetes. *Exp Diabetes Res*, 2012; 2012: 502654
- 29) Stanley TL, Zanni MV, Johnsen S, Rasheed S, Makimura H, Lee H, Khor VK, Ahima RS, Grinspoon SK: TNF- α antagonism with etanercept decreases glucose and increases the proportion of high molecular weight adiponectin in obese subjects with features of the metabolic syndrome. *J Clin Endocrinol Metab*, 2011; 96: E146-150
- 30) Rose-John S: ADAM17, shedding, TACE as therapeutic targets. *Pharmacol Res*, 2013; 71: 19-22
- 31) Monroy A, Kamath S, Chavez AO, Centonze VE, Veerasamy M, Barrentine A, Wewer JJ, Coletta DK, Jenkinson C, Jhingan RM, Smokler D, Reyna S, Musi N, Khokha R, Federici M, Tripathy D, DeFronzo RA, Folli F: Impaired regulation of the TNF- α converting enzyme/tissue inhibitor of metalloproteinase 3 proteolytic system in skeletal muscle of obese type 2 diabetic patients: a new mechanism of insulin resistance in humans. *Diabetologia*, 2009; 52: 2169-2181
- 32) Menghini R, Fiorentino L, Casagrande V, Lauro R, Federici M: The role of ADAM17 in metabolic inflammation. *Atherosclerosis*, 2013; 228: 12-17
- 33) Menghini R, Casagrande V, Menini S, Marino A, Marzano V, Hribal ML, Gentileschi P, Lauro D, Schillaci O, Pugliese G, Sbraccia P, Urbani A, Lauro R, Federici M: TIMP3 overexpression in macrophages protects from insulin resistance, adipose inflammation, and nonalcoholic fatty liver disease in mice. *Diabetes*, 2012; 61: 454-462
- 34) Casagrande V, Menghini R, Menini S, Marino A, Marchetti V, Cavalera M, Fabrizi M, Hribal ML, Pugliese G, Gentileschi P, Schillaci O, Porzio O, Lauro D, Sbraccia P, Lauro R, Federici M: Overexpression of tissue inhibitor of metalloproteinase 3 in macrophages reduces atherosclerosis in low-density lipoprotein receptor knockout mice. *Arterioscler Thromb Vasc Biol*, 2012; 32: 74-81
- 35) McIlwain DR, Lang PA, Maretzky T, Hamada K, Ohishi K, Maney SK, Berger T, Murthy A, Duncan G, Xu HC, Lang KS, Haussinger D, Wakeham A, Itie-Youten A, Khokha R, Ohashi PS, Blobel CP, Mak TW: iRhom2 regulation of TACE controls TNF-mediated protection against *Listeria* and responses to LPS. *Science*, 2012; 335: 229-232
- 36) Adrain C, Zettl M, Christova Y, Taylor N, Freeman M: Tumor necrosis factor signaling requires iRhom2 to promote trafficking and activation of TACE. *Science*, 2012; 335: 225-228
- 37) Cardellini M, Menghini R, Martelli E, Casagrande V, Marino A, Rizza S, Porzio O, Mauriello A, Solini A, Ippoliti A, Lauro R, Folli F, Federici M: TIMP3 is reduced in atherosclerotic plaques from subjects with type 2 diabetes and increased by SirT1. *Diabetes*, 2009; 58: 2396-2401
- 38) Szalad A, Katakowski M, Zheng X, Jiang F, Chopp M: Transcription factor Sp1 induces ADAM17 and contributes to tumor cell invasiveness under hypoxia. *J Exp Clin Cancer Res*, 2009; 28: 129
- 39) Mizui Y, Yamazaki K, Sagane K, Tanaka I: cDNA cloning of mouse tumor necrosis factor- α converting enzyme (TACE) and partial analysis of its promoter. *Gene*, 1999; 233: 67-74

Supplementary Fig. 1. Primary polymorphisms and putative transcription factors in the 5'-regulatory region of the *ADAM17* gene. The positions of the nine variations of the *ADAM17* promoter are depicted, and the polymorphisms analyzed in the present study are in bold. The bold arrows represent the putative transcription factors, according to previous publications^{38, 39}.

Supplementary Table 1. Comparison of baseline characteristics with respect to the rs11684747 genotypes and alleles between the IS patient and control groups

Characteristics	rs11684747 IS patient group					rs11684747 control group					P _G	P _A
	Genotype n (%)			Allele n (%)		Genotype n (%)			Allele n (%)			
	AA	GA	GG	A	G	AA	GA	GG	A	G		
Age												
<70 years	142 (95.30)	6 (4.03)	1 (0.67)	290 (97.32)	8 (2.68)	133 (96.38)	5 (3.62)	0	271 (98.19)	5 (1.81)	0.617	0.482
≥70 years	185 (95.85)	8 (4.15)	0	378 (97.93)	8 (2.07)	150 (94.94)	8 (5.06)	0	308 (97.47)	8 (2.53)	0.682	0.685
Gender												
Male	217 (95.18)	10 (4.39)	1 (0.43)	445 (97.59)	11 (2.41)	181 (96.28)	7 (3.72)	0	369 (98.14)	7 (1.86)	0.622	0.587
Female	110 (96.49)	4 (3.51)	0	224 (98.25)	4 (1.75)	102 (94.44)	6 (5.56)	0	210 (97.22)	6 (2.78)	0.462	0.468
Hypertension												
Yes	184 (95.58)	7 (3.87)	1 (0.55)	368 (98.13)	9 (2.40)	62 (95.38)	3 (4.62)	0	127 (97.69)	3 (2.31)	0.796	0.959
No	143 (95.33)	7 (4.67)	0	293 (97.67)	6 (2.33)	221 (95.67)	10 (4.33)	0	456 (97.84)	10 (2.16)	0.876	0.896
Smoking												
Yes	163 (94.22)	9 (5.2)	1 (0.58)	335 (96.82)	11 (3.18)	52 (96.3)	2 (3.7)	0	106 (98.15)	2 (1.85)	0.770	0.470
No	164 (97.04)	5 (2.96)	0	333 (98.52)	5 (1.48)	231 (95.45)	11 (4.55)	0	473 (97.73)	11 (2.27)	0.413	0.418
Diabetes												
Yes	68 (97.14)	2 (2.86)	0	138 (98.57)	2 (1.43)	33 (100)	0	0	66 (100)	0	0.198	0.329
No	259 (95.22)	12 (4.41)	1 (0.37)	530 (97.43)	13 (2.57)	250 (95.06)	13 (4.94)	0	515 (97.91)	13 (2.47)	0.592	0.942

P_G: *p* value of the difference in genotype between the cases and controls

P_A: *p* value of the difference in alleles between the cases and controls

Supplementary Table 2. Comparison of baseline characteristics with respect to the rs11689958 genotypes and alleles between the IS patient and control groups

Characteristics	rs11689958 IS patient group					rs11689958 control group					P _G	P _A
	Genotype n (%)			Allele n (%)		Genotype n (%)			Allele n (%)			
	GG	GA	AA	G	A	GG	GA	AA	G	A		
Age												
< 70 years	142 (95.30)	6 (4.03)	1 (0.67)	290 (97.32)	8 (2.68)	133 (96.38)	5 (3.62)	0	271 (98.19)	5 (1.81)	0.617	0.482
≥ 70 years	185 (95.85)	8 (4.15)	0	378 (97.93)	8 (2.07)	150 (94.94)	8 (5.06)	0	308 (97.47)	8 (2.53)	0.682	0.685
Gender												
Male	217 (95.18)	10 (4.39)	1 (0.43)	445 (97.59)	11 (2.41)	181 (96.28)	7 (3.72)	0	369 (98.14)	7 (1.86)	0.622	0.587
Female	110 (96.49)	4 (3.51)	0	224 (98.25)	4 (1.75)	102 (94.44)	6 (5.56)	0	210 (97.22)	6 (2.78)	0.462	0.468
Hypertension												
Yes	184 (95.58)	7 (3.87)	1 (0.55)	368 (98.13)	9 (2.40)	62 (95.38)	3 (4.62)	0	127 (97.69)	3 (2.31)	0.756	0.959
No	143 (95.33)	7 (4.67)	0	293 (97.67)	6 (2.33)	221 (95.67)	10 (4.33)	0	456 (97.84)	10 (2.16)	0.876	0.896
Smoking												
Yes	163 (94.22)	9 (5.2)	1 (0.58)	335 (96.82)	11 (3.18)	52 (96.3)	2 (3.7)	0	106 (98.15)	2 (1.85)	0.770	0.470
No	164 (97.04)	5 (2.96)	0	333 (98.52)	5 (1.48)	231 (95.45)	11 (4.55)	0	473 (97.73)	11 (2.27)	0.413	0.418
Diabetes												
Yes	68 (97.14)	2 (2.86)	0	138 (98.57)	2 (1.43)	33 (100)	0	0	66 (100)	0	0.198	0.329
No	259 (95.22)	12 (4.41)	1 (0.37)	530 (97.43)	13 (2.57)	250 (95.06)	13 (4.94)	0	515 (97.91)	13 (2.47)	0.592	0.942

P_G: *p* value of the difference in genotype between the cases and controlsP_A: *p* value of the difference in alleles between the cases and controls**Supplementary Table 3.** Comparison of baseline characteristics with respect to the rs12692386 genotypes and alleles between the IS patient and control groups

Characteristics	rs12692386 IS patient group					rs12692386 control group					P _A	P _G
	Genotype n (%)			Allele n (%)		Genotype n (%)			Allele n (%)			
	AA	GA	GG	A	G	AA	GA	GG	A	G		
Age												
< 70 years	94 (63.09)	49 (32.88)	6 (4.03)	237 (79.53)	61 (20.47)	83 (60.15)	50 (36.23)	5 (3.62)	216 (78.26)	60 (21.74)	0.833	0.710
≥ 70 years	109 (56.48)	74 (38.34)	10 (5.18)	292 (75.65)	94 (24.35)	89 (56.33)	64 (40.51)	5 (3.16)	242 (76.58)	74 (23.42)	0.628	0.773
Gender												
Male	132 (57.89)	87 (38.16)	9 (3.95)	351 (76.97)	105 (23.13)	110 (58.51)	72 (38.3)	6 (3.19)	292 (77.66)	84 (24.34)	0.918	0.814
Female	71 (62.28)	36 (31.58)	7 (6.14)	178 (78.07)	50 (21.93)	62 (57.41)	42 (38.89)	4 (3.70)	166 (76.86)	50 (22.94)	0.422	0.759
Hypertension												
Yes	112 (58.33)	71 (36.98)	9 (4.69)	295 (76.82)	89 (23.18)	44 (67.69)	19 (29.23)	2 (3.08)	107 (82.31)	23 (17.69)	0.401	0.190
No	91 (60.67)	52 (34.67)	7 (4.66)	234 (78.00)	66 (22.00)	128 (55.41)	95 (41.13)	8 (3.46)	351 (75.97)	111 (24.03)	0.416	0.518
Smoking												
Yes	102 (58.96)	60 (34.68)	11 (6.36)	264 (76.30)	82 (23.70)	38 (70.37)	15 (27.78)	1 (1.85)	89 (84.26)	17 (15.74)	0.218	0.095
No	101 (59.76)	63 (37.28)	5 (2.96)	265 (78.4)	73 (21.6)	134 (55.37)	99 (40.91)	9 (3.72)	367 (75.83)	117 (24.17)	0.658	0.389
Diabetes												
Yes	39 (55.71)	25 (35.71)	6 (8.57)	103 (73.57)	37 (26.43)	19 (57.58)	10 (30.30)	4 (12.12)	48 (72.73)	18 (27.27)	0.780	0.898
No	164 (60.29)	98 (36.03)	10 (3.68)	426 (78.31)	118 (21.69)	153 (58.17)	104 (49.55)	6 (2.28)	410 (77.95)	116 (22.05)	0.494	0.886

P_A: *p* value of the difference in genotype between the cases and controlsP_G: *p* value of the difference in alleles between the cases and controls

Supplementary Table 4. Comparison of baseline characteristics with respect to the rs55790676 genotypes and alleles between the IS patient and control groups

Characteristics	rs55790676 IS patient group					rs55790676 control group					P _G	P _T
	Genotype n (%)			Allele n (%)		Genotype n (%)			Allele n (%)			
	GG	GT	TT	G	T	GG	GT	TT	G	T		
Age												
<70 years	142 (95.30)	6 (4.03)	1 (0.67)	290 (97.32)	8 (2.68)	133 (96.38)	5 (3.62)	0	271 (98.19)	5 (1.81)	0.617	0.482
≥70 years	185 (95.85)	8 (4.15)	0	378 (97.93)	8 (2.07)	150 (94.94)	8 (5.06)	0	308 (97.47)	8 (2.53)	0.682	0.685
Gender												
Male	217 (95.18)	10 (4.39)	1 (0.43)	445 (97.59)	11 (2.41)	181 (96.28)	7 (3.72)	0	369 (98.14)	7 (1.86)	0.622	0.587
Female	110 (96.49)	4 (3.51)	0	224 (98.25)	4 (1.75)	102 (94.44)	6 (5.56)	0	210 (97.22)	6 (2.78)	0.462	0.468
Hypertension												
Yes	184 (95.58)	7 (3.87)	1 (0.55)	368 (98.13)	9 (2.40)	62 (95.38)	3 (4.62)	0	127 (97.69)	3 (2.31)	0.796	0.959
No	143 (95.33)	7 (4.67)	0	293 (97.67)	6 (2.33)	221 (95.67)	10 (4.33)	0	456 (97.84)	10 (2.16)	0.876	0.896
Smoking												
Yes	163 (94.22)	9 (5.2)	1 (0.58)	335 (96.82)	11 (3.18)	52 (96.3)	2 (3.7)	0	106 (98.15)	2 (1.85)	0.770	0.470
No	164 (97.04)	5 (2.96)	0	333 (98.52)	5 (1.48)	231 (95.45)	11 (4.55)	0	473 (97.73)	11 (2.27)	0.413	0.418
Diabetes												
Yes	68 (97.14)	2 (2.86)	0	138 (98.57)	2 (1.43)	33 (100)	0	0	66 (100)	0	0.198	0.329
No	259 (95.22)	12 (4.41)	1 (0.37)	530 (97.43)	13 (2.57)	250 (95.06)	13 (4.94)	0	515 (97.91)	13 (2.47)	0.592	0.942

P_G: *p* value of the difference in genotype between the cases and controlsP_T: *p* value of the difference in alleles between the cases and controls**Supplementary Table 5.** Comparison of baseline characteristics with respect to the rs1524668 genotypes and alleles between the IS patient and control groups

Characteristics	rs1524668 IS patient group					rs1524668 control group					P _A	P _C
	Genotype n (%)			Allele n (%)		Genotype n (%)			Allele n (%)			
	AA	AC	CC	A	C	AA	AC	CC	A	C		
Age												
<70 years	117 (78.52)	32 (21.48)	0	266 (89.26)	32 (10.74)	111 (80.43)	26 (18.85)	1 (0.72)	248 (89.86)	28 (10.14)	0.507	0.816
≥70 years	143 (74.10)	47 (24.35)	3 (1.55)	333 (86.27)	53 (13.73)	119 (75.32)	39 (24.68)	0	277 (88.29)	37 (11.71)	0.290	0.444
Gender												
Male	170 (74.56)	55 (24.12)	3 (1.32)	395 (86.62)	61 (13.38)	146 (77.66)	41 (21.81)	1 (0.53)	333 (88.56)	43 (11.44)	0.598	0.399
Female	90 (78.95)	24 (21.05)	0	204 (89.47)	24 (10.53)	84 (77.78)	24 (22.22)	0	192 (88.89)	24 (11.01)	0.832	0.843
Hypertension												
Yes	145 (75.52)	46 (23.96)	1 (0.52)	336 (87.50)	48 (12.50)	51 (78.46)	14 (21.54)	0	116 (89.23)	14 (10.77)	0.773	0.600
No	115 (76.67)	33 (22.00)	2 (1.33)	263 (87.67)	37 (12.33)	179 (77.49)	51 (22.08)	1 (0.43)	409 (88.53)	53 (11.47)	0.624	0.719
Smoking												
Yes	130 (75.15)	40 (23.12)	3 (1.73)	300 (86.71)	46 (13.29)	43 (79.63)	11 (20.37)	0	97 (89.81)	11 (10.19)	0.553	0.395
No	130 (76.92)	39 (23.08)	0	299 (88.46)	39 (11.54)	187 (77.27)	54 (22.31)	1 (0.42)	428 (88.43)	56 (11.57)	0.696	0.989
Diabetes												
Yes	58 (82.86)	12 (17.14)	0	128 (91.43)	12 (8.57)	25 (75.76)	7 (21.21)	1 (3.03)	57 (86.36)	9 (13.64)	0.292	0.262
No	202 (74.55)	67 (24.36)	3 (1.09)	471 (86.58)	73 (13.43)	205 (77.95)	58 (22.05)	0	468 (88.97)	58 (11.03)	0.172	0.233

P_A: *p* value of the difference in genotype between the cases and controlsP_C: *p* value of the difference in alleles between the cases and controls