Genetic risk for atherothrombotic cerebral infarction in individuals stratified by sex or conventional risk factors for atherosclerosis

SACHIYO YAMAGUCHI[1], YOSHIJI YAMADA[1,2], NORIFUMI METOKI[3], HIDEMI YOSHIDA[4], KEI SATOH[4], SAHOKO ICHIHARA[1], KIMIHIKO KATO[5], TAKASHI KAMEYAMA[5], KIYOSHI YOKOI[5], HITOSHI MATSUO[6], TOMONORI SEGAWA[6], SACHIRO WATANABE[6] and YOSHINORI NOZAWA[2]

[1]Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu; [2]Gifu International Institute of Biotechnology, Kakamigahara, Japan; [3]Department of Internal Medicine, Hirosaki Stroke Center, Hirosaki; [4]Department of Vascular Biology, Institute of Brain Science, Hirosaki University School of Medicine, Hirosaki; [5]Department of Cardiovascular Medicine and Department of Neurology, Gifu Prefectural Tajimi Hospital, Tajimi; [6]Department of Cardiology, Gifu Prefectural Gifu Hospital, Gifu, Japan

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Abstract. The aim of the present study was to assess the genetic risk for atherothrombotic cerebral infarction (ACI) in men and women separately as well as in individuals with or without conventional risk factors for atherosclerosis and thereby to contribute to the personalized prevention of ACI. The study population comprised 2705 unrelated Japanese individuals (1244 men, 1461 women), including 636 subjects (372 men, 264 women) with ACI. Subjects with ACI and controls either had or did not have conventional risk factors for atherosclerosis, including hypertension, hypercholesterolemia, and diabetes mellitus. The genotypes for 202 polymorphisms of 152 candidate genes were determined by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. Multivariable logistic regression analysis and a stepwise forward selection procedure revealed that 11 different polymorphisms were significantly ($P<0.005$) associated with ACI in women or men or in individuals with or without hypertension, hypercholesterolemia, or diabetes mellitus: the 584C→T polymorphism of *LIPG*, 5665G→T of *EDN1*, and G→A of *CCL11* in women; 677C→T of *MTHFR*, 1323C→T of *ITGB2*, 3932T→C of *APOE*, and -231A→G of *EDNRA* in men; -572 G→C of *IL6* in hypertensive individuals; -403G→A of *CCL5* and G→A of *COMT* in individuals with hypercholesterolemia; and 3932T→C of *APOE* and A→G of *TNFSF4* in diabetic individuals. Polymorphisms associated with ACI may thus differ between women and men as well as among individuals with different risk factors. Stratification of subjects on the basis of sex or conventional risk factors for atherosclerosis may therefore be important in order to achieve the personalized prevention of ACI with the use of genetic information.

Correspondence to: Dr Yoshiji Yamada, Department of Human Functional Genomics, Life Science Research Center, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan
E-mail: yamada@gene.mie-u.ac.jp

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Introduction

Stroke is the third most common cause of death after cancer and heart disease and is the leading cause of acquired disability in adults (1). In the United States, approximately 700,000 individuals suffer a new or recurrent stroke and nearly 160,000 die from stroke-related causes each year. The total number of individuals who have experienced a stroke is 5.5 million (2). In Japan, the prevalence of stroke is 1.4 million, with nearly 132,000 deaths from this condition occurring each year (Ministry of Health, Labor, and Welfare of Japan).

Ischemic stroke, which accounts for approximately 80% of all strokes, is a complex disorder. The main cause of ischemic stroke is atherothrombosis, with the principal and treatable risk factors including hypertension, hypercholesterolemia, and diabetes mellitus (3). In addition to these conventional risk factors, genetic variants are important in the pathogenesis of ischemic stroke (4,5). Recent genetic epidemiological studies have thus identified several genes related to the prevalence of stroke, including those for interleukin-6 (6,7), methylenetetrahydrofolate reductase (8,9), paraoxonase (10), phosphodiesterase 4D (11), 5-lipoxygenase activating protein (12), and cyclooxygenase 2 (13). However, the genetic determinants of ischemic stroke remain largely unknown.

We hypothesized that gene polymorphisms related to atherothrombotic cerebral infarction (ACI) might differ between women and men as well as among individuals with or without conventional risk factors for atherosclerosis. We have therefore performed an association study of 202

Table I. Characteristics of male and female subjects with ACI and controls.

Characteristic	Women			Men		
	ACI	controls	P	ACI	controls	P
No. of subjects	264	1197		372	872	
Age (years)	68.6±12.2	62.8±12.0	<0.0001	66.3±10.2	61.7±12.0	<0.0001
BMI (kg/m^2)	23.7±3.5	23.5±3.2	0.3200	23.0±2.8	23.3±2.8	0.0780
Smoking (%)	1.9	3.2	0.2400	22.6	32.5	0.0004
Hypertension (%)	72.7	35.8	<0.0001	66.9	45.3	<0.0001
Hypercholesterolemia (%)	39.4	32.3	0.0275	30.9	23.1	0.0068
Diabetes mellitus (%)	33.7	15.9	<0.0001	42.7	22.4	<0.0001

Data for age and BMI are means ± SD.

polymorphisms and ACI for women and men separately as well as for individuals with or without hypertension, hypercholesterolemia, or diabetes mellitus. Our aim was to assess the genetic risk for ACI in men and women as well as in individuals with or without conventional risk factors for atherosclerosis and thereby to contribute to the personalized prevention of ACI.

Materials and methods

Study population. The study population comprised 2705 unrelated Japanese individuals (1244 men, 1461 women) who either visited outpatient clinics of or were admitted to one of the participating hospitals (Gifu Prefectural Gifu, Tajimi, and Gero Hot Spring Hospitals; Hirosaki University Hospital; Reimeikyo Rehabilitation Hospital; and Yokohama General Hospital) between October 2002 and March 2005. A total of 636 consecutive subjects (372 men, 264 women) with ACI was enrolled in the study. The diagnosis of ischemic stroke was based on the occurrence of a new and abrupt focal neurological deficit, with neurological symptoms and signs persisting for more than 24 h; it was confirmed by positive findings in computed tomography or magnetic resonance imaging (or both) of the head. The type of stroke was determined according to the Classification of Cerebrovascular Diseases III (14). Individuals with cardiogenic embolic infarction, lacunar infarction, transient ischemic attack, hemorrhagic stroke, cerebrovascular malformations, brain tumors, or traumatic cerebrovascular diseases were excluded from the study, as were those with atrial fibrillation in the absence or presence of valvular heart disease.

The 2069 control subjects (872 men, 1197 women) visited outpatient clinics of the participating hospitals for an annual health checkup. They had no history of ischemic or hemorrhagic stroke or other cerebral diseases; of coronary heart disease, peripheral arterial occlusive disease, or other atherosclerotic diseases; or of other thrombotic, embolic, or hemorrhagic disorders.

Subjects with ACI and controls either had or did not have conventional risk factors for atherosclerosis, including hypertension (systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg, or both), hypercholesterolemia (serum total cholesterol of ≥5.72 mmol/l), diabetes mellitus (fasting blood glucose of ≥6.93 mmol/l or hemoglobin A_{1c} of ≥6.5%, or both), obesity [body mass index (BMI) of ≥25 kg/m^2], and cigarette smoking (≥10 cigarettes daily). The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University School of Medicine, Hirosaki University School of Medicine, Gifu International Institute of Biotechnology, and participating hospitals. Written informed consent was obtained from each participant.

Selection of polymorphisms. With the use of public databases, we selected 152 candidate genes that might be associated with ACI on the basis of a comprehensive overview of vascular biology; platelet function; leukocyte, lymphocyte, and monocyte-macrophage biology; coagulation and fibrinolysis cascades; neurological factors; as well as lipid, glucose, and homocysteine metabolism and other metabolic factors. We further selected 202 polymorphisms of these genes, most located in the promoter region, exons, or splice donor or acceptor sites of introns, that might be expected to result in changes in the function or expression of the encoded protein (15).

Genotyping of polymorphisms. Venous blood (7 ml) was collected into tubes containing 50 mmol/l EDTA (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 202 polymorphisms were determined (G&G Science, Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with the use of suspension array technology (Luminex 100; Luminex, Austin, TX). Detailed methodology for genotyping was described previously (16).

Statistical analysis. Clinical data were compared between subjects with ACI and controls by the unpaired Student's t-test. Qualitative data were compared by the Chi-square test. Allele frequencies were estimated by the gene counting method, and the Chi-square test was used to identify departure from Hardy-

Table II. Multivariable logistic regression analysis of polymorphisms related to ACI in women.

Gene	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
LIPG	584C→T (Thr111Ile)	**0.0005**	1.66 (1.25-2.21)	0.1356		**0.0012**	1.63 (1.21-2.19)	0.0335	1.93 (1.03-3.50)
CCL11	G→A (Ala23Thr)	**0.0008**	0.53 (0.36-0.76)	0.2374		**0.0016**	0.54 (0.37-0.78)	0.1944	
EDN1	5665G→T (Lys198Asn)	**0.0003**	1.69 (1.27-2.25)	0.1755		**0.0008**	1.67 (1.24-2.25)	0.0288	1.81 (1.05-3.04)
AKAP10	A→G (Ile646Val)	0.0080	0.66 (0.49-0.90)	0.0537		0.0276	0.70 (0.51-0.96)	0.0333	0.32 (0.09-0.82)
UTS2	G→A (Ser89Asn)	0.0175	1.42 (1.06-1.89)	0.2085		0.0058	1.15 (1.13-2.03)	0.3707	
IL6	-572G→C	0.2590		**0.0033**	1.56 (1.16-2.10)	0.7415		0.1198	
PTGDS	4111A→C	0.4035		0.0164	0.70 (0.52-0.94)	0.1808		0.5660	
ANXA5	-1C→T	0.1830		0.7426		0.3885		0.7417	
KCNJ11	A→G (Glu23Lys)	0.0058	1.93 (1.23-3.16)	0.0932		0.0154	1.83 (0.51-0.96)	0.0040	2.08 (1.28-3.48)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, BMI, and the prevalence of smoking, hypertension, hypercholesterolemia, and diabetes mellitus. P values of <0.005 are shown in bold.

Weinberg equilibrium. In the initial screen, the genotype distribution of each autosomal polymorphism was compared by the Chi-square test (3x2) between subjects with ACI and controls. For gene polymorphisms located on the X chromosome, allele frequencies were compared by the Chi-square test (2x2). Polymorphisms related (P<0.05) to ACI were further examined by multivariable logistic regression analysis with adjustment for covariates (with the exception of that used for stratification of subjects), with ACI as a dependent variable and independent variables including age, sex (0: woman; 1: man), BMI, smoking status (0: nonsmoker; 1: smoker), metabolic variables (0: no history of hypertension, diabetes mellitus, or hypercholesterolemia; 1: positive history), and genotype of each polymorphism. Each genotype was assessed according to dominant, recessive, and additive (additive 1 and 2) genetic models, and the P value, odds ratio, and 95% confidence interval were calculated. Additive genetic models comprised two groups: heterozygotes versus wild-type homozygotes for the additive 1 model, and variant homozygotes versus wild-type homozygotes for the additive 2 model. We also performed a stepwise forward selection procedure to examine the effects of genotypes as well as of other covariates on ACI. Given the multiple comparisons of genotypes with ACI, we adopted the criterion of P<0.005 for significant association. For other clinical background data, a P value of <0.05 was considered statistically significant. Statistical significance was examined by two-sided tests, and statistical analyses were performed with JMP software version 5.1 (SAS Institute, Cary, NC).

Results

Association of polymorphisms with ACI in women or men. Characteristics of female and male subjects are shown in Table I. Among women, age and the prevalence of hypertension, hypercholesterolemia, and diabetes mellitus were greater in subjects with ACI than in controls. Among men, age and the prevalence of hypertension, hypercholesterolemia, and diabetes mellitus were greater, whereas the prevalence of smoking was lower, in subjects with ACI than in controls. The Chi-square test revealed that nine and 16 polymorphisms were related (P<0.05) to ACI in women and men, respectively (Supplementary Table I). These polymorphisms were further analyzed for their possible association with ACI. Multivariable logistic regression analysis with adjustment for age, BMI, and the prevalence of smoking, hypertension, hypercholesterolemia, and diabetes mellitus revealed that the 584C→T polymorphism of *LIPG* (dominant and additive 1 models), the G→A polymorphism of *CCL11* (dominant and additive 1 models), the 5665G→T polymorphism of *EDN1* (dominant and additive 1 models), the -572G→C polymorphism of *IL6* (recessive model), and the A→G polymorphism of *KCNJ11* (additive 2 model) were significantly (P<0.005) associated with ACI for woman (Table II). For men, the 677C→T polymorphism of *MTHFR* (recessive and additive 2 models), the 1323C→T polymorphism of *ITGB2* (dominant model), the T→G polymorphism of *THBS2* (additive 1 model), the 3932T→C polymorphism of *APOE* (dominant and additive 1 models), and the -231A→G polymorphism of *EDNRA* (recessive model) were significantly associated with ACI (Table III). We also performed a stepwise forward selection procedure to examine the effects of genotypes for the genes related to ACI as well as of age, BMI, smoking, hypertension, hypercholesterolemia, and diabetes mellitus on the prevalence of ACI (Table IV). Each genotype was examined according to a dominant or recessive model on the basis of statistical significance in the multivariable logistic regression analysis. In descending order of statistical significance, hypertension, age, *LIPG* genotype (dominant model), *EDN1* genotype (dominant model), *CCL11* genotype (dominant model), and diabetes mellitus significantly and independently (P<0.005) affected the prevalence of ACI in women. In men, diabetes mellitus, age, hypertension, *MTHFR* genotype (recessive model), *ITGB2* genotype (dominant model), *APOE* genotype (dominant model), and *EDNRA* genotype (recessive model) significantly and independently affected the prevalence of ACI.

Table III. Multivariable logistic regression analysis of polymorphisms related to ACI in men.

Gene	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
MTHFR	677C→T (Ala222Val)	0.5927		**0.0002**	1.85 (1.33-2.55)	0.4220		**0.0030**	1.73 (1.20-2.48)
ITGB2	1323C→T	**0.0016**	1.51 (1.17-1.95)	0.0882		0.0051	1.46 (1.12-1.91)	0.0218	1.83 (1.08-3.06)
THBS2	T→G (3' UTR)	0.0120	1.52 (1.09-2.09)	0.4171		**0.0049**	1.61 (1.15-2.25)	0.5019	
ALOX5	G→A (Glu254Lys)			0.0512		0.0512			
IPF1	-108/3G→4G	0.0115	0.69 (0.51-0.92)	0.6660		0.0114	0.67 (0.49-0.91)	0.0699	
APOE	3932T→C (Cys112Arg)	**0.0021**	1.62 (1.19-2.20)	0.6337		**0.0024**	1.62 (1.19-2.22)	0.5415	
APOE	-219G→T	0.0121	2.02 (1.19-3.60)	0.2617		0.0203	1.96 (1.13-3.56)	0.0112	2.07 (1.20-3.73)
KCNJ11	A→G (Glu23Lys)	0.0480	0.70 (0.50-1.00)	0.0251	0.74 (0.57-0.96)	0.2051		0.0134	0.62 (0.42-0.91)
ACDC	-11,377C→G	0.0286	1.33 (1.03-1.72)	0.0453	1.66 (1.00-2.73)	0.0950		0.0211	1.82 (1.09-3.03)
APOA1	84T→C	0.0505		0.0101	1.98 (1.17-3.32)	0.2100		0.0057	2.11 (1.24-3.59)
APOH	341G→A (Ser88Asn)			0.1745		0.1745			
SELE	561A→C (Ser128Arg)	0.0236	1.73 (1.07-2.91)	0.1020		0.0573		0.0964	
TNFSF4	A→G	0.4555		0.0053	3.94 (1.51-10.67)	0.9612		0.0053	3.94 (1.51-10.71)
F7	11,496G→A (Arg353Gln)	0.3265		0.0898		0.1606		0.0985	
UCP3	-55C→T	0.5552		0.1807		0.2658		0.3490	
EDNRA	-231A→G	0.2303		**0.0027**	0.65 (0.48-0.86)	0.8513		0.0146	0.63 (0.44-0.91)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, BMI, and the prevalence of smoking, hypertension, hypercholesterolemia, and diabetes mellitus. P values of <0.005 are shown in bold.

Table IV. Effects of genotypes and other characteristics on ACI for women or men as determined by a stepwise forward selection procedure.

Women			Men		
Variable	P	R^2	Variable	P	R^2
Hypertension	<0.0001	0.0876	Diabetes mellitus	<0.0001	0.0337
Age	<0.0001	0.0158	Age	<0.0001	0.0210
LIPG (*CC* versus *TT+CT*)	0.0002	0.0102	Hypertension	<0.0001	0.0133
EDN1 (*GG* versus *TT+GT*)	0.0002	0.0099	*MTHFR* (*CC+CT* versus *TT*)	0.0003	0.0087
CCL11 (*GG* versus *AA+GA*)	0.0003	0.0094	*ITGB2* (*CC* versus *TT+CT*)	0.0019	0.0064
Diabetes mellitus	0.0014	0.0074	*APOE* (*TT* versus *CC+TC*)	0.0021	0.0062
AKAP10 (*AA* versus *GG+AG*)	0.0060	0.0055	*EDNRA* (*AA+AG* versus *GG*)	0.0045	0.0053
UTS2 (*GG* versus *AA+GA*)	0.0062	0.0054	Smoking	0.0060	0.0050
KCNJ11 (*AA* versus *GG+AG*)	0.0068	0.0053	*APOA1* (*TT+TC* versus *CC*)	0.0078	0.0047
IL6 (*GG+GC* versus *CC*)	0.0070	0.0053	*TNFSF4* (*AA+AG* versus *GG*)	0.0083	0.0046
PTGDS (*AA+AC* versus *CC*)	0.0101	0.0048	*KCNJ11* (*AA+AG* versus *GG*)	0.0165	0.0038
			THBS2 (*TT* versus *GG+TG*)	0.0173	0.0037
			APOE (*GG* versus *TT+GT*)	0.0233	0.0034
			IPF1 (*3G3G* versus *4G4G+3G4G*)	0.0296	0.0031
			BMI	0.0361	0.0029
			ACDC (*CC* versus *GG+CG*)	0.0449	0.0027

R^2, contribution rate.

Association of polymorphisms with ACI in the absence or presence of hypertension. Characteristics of individuals with or without hypertension are shown in Table V. Among normotensive individuals, age and the prevalence of diabetes mellitus

Table V. Characteristics of subjects with ACI and controls according to the absence or presence of hypertension.

Characteristic	Hypertension (-)			Hypertension (+)		
	ACI	Controls	P	ACI	Controls	P
No. of subjects	195	1245		441	824	
Age (years)	66.8±12.9	60.1±12.2	<0.0001	67.4±10.3	65.6±11.0	0.0040
BMI (kg/m^2)	23.3±2.5	23.3±2.8	0.9680	23.3±0.2	23.6±0.1	0.1480
Smoking (%)	13.3	15.9	0.3487	14.3	14.9	0.7584
Hypercholesterolemia (%)	14.9	22.5	0.0124	43.1	37.7	0.0647
Diabetes mellitus (%)	20.0	10.8	0.0006	47.4	30.3	<0.0001

Data for age and BMI are means ± SD.

Table VI. Multivariable logistic regression analysis of polymorphisms related to ACI in hypertensive individuals.

Gene	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
IL6	-572G→C	0.4823		**0.0021**	1.47 (1.14-1.87)	0.8442		0.2282	
SLC26A8	A→G (Ile639Val)	0.0270	1.31 (1.03-1.66)	0.8149		0.0167	1.35 (1.06-1.74)	0.7254	
GP6	13,254T→C (Ser219Pro)	0.0231	1.90 (1.09-3.31)			0.0231	1.90 (1.09-3.32)		
THBS2	T→G (3' UTR)	0.0262	1.41 (1.04-1.89)	0.2138		0.0476	1.37 (1.00-1.86)	0.1785	
TNFSF4	A→G	0.2489		0.0224	3.28 (1.22-9.75)	0.5264		0.0205	3.35 (1.24-9.96)
PCSK9	23,968A→G (Glu670Gly)	0.0172	1.67 (1.09-2.54)	0.5640		0.0106	1.75 (1.14-2.69)	0.5925	
IPF1	-108/3G→4G	0.0217	0.72 (0.55-0.95)	0.5605		0.0263	0.72 (0.53-0.96)	0.0700	
CCR5	59,029G→A	0.5091		0.0329	1.32 (1.02-1.71)	0.1409		0.4913	
SELE	561A→C (Ser128Arg)	0.4518		0.7091		0.7499		0.7089	
HNF4A	A→G	0.9618		0.0226	1.33 (1.04-1.69)	0.4980		0.6268	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, BMI, and the prevalence of smoking, hypercholesterolemia, and diabetes mellitus. P values of <0.005 are shown in bold.

Table VII. Effects of genotypes and other characteristics on ACI in the absence or presence of hypertension as determined by a stepwise forward selection procedure.

Hypertension (-)			Hypertension (+)		
Variable	P	R^2	Variable	P	R^2
Age	<0.0001	0.0432	Diabetes mellitus	<0.0001	0.0215
Sex	<0.0001	0.0383	*IL6* (*GG+GC* versus *CC*)	0.0016	0.0061
Smoking	0.0032	0.0076	*SLC26A8* (*AA* versus *GG+AG*)	0.0081	0.0043
SAH (*AA* versus *GG+AG*)	0.0082	0.0061	Age	0.0112	0.0039
Diabetes mellitus	0.0123	0.0055	*HNF4A* (*AA+AG* versus *GG*)	0.0120	0.0039
MTHFR (*CC+CT* versus *TT*)	0.0131	0.0054	*TNFSF4* (*AA+AG* versus *GG*)	0.0133	0.0038
AGTR1 (*AA+AC* versus *CC*)	0.0166	0.0050	Sex	0.0168	0.0035
			THBS2 (*TT* versus *GG+TG*)	0.0200	0.0033
			GP6 (*TT* versus *CC+TC*)	0.0201	0.0033
			IPF1 (*3G3G* versus *4G4G+3G4G*)	0.0264	0.0030
			PCSK9 (*AA* versus *GG+AG*)	0.0340	0.0028
			CCR5 (*GG+GA* versus *AA*)	0.0493	0.0024

R^2, contribution rate.

Table VIII. Characteristics of subjects with ACI and controls according to the absence or presence of hypercholesterolemia.

Characteristic	Hypercholesterolemia (-)			Hypercholesterolemia (+)		
	ACI	Controls	P	ACI	Controls	P
No. of subjects	417	1478		219	591	
Age (years)	68.1±11.6	62.0±12.3	<0.0001	65.6±10.1	62.6±11.3	<0.0001
BMI (kg/m^2)	23.2±2.9	23.3±3.0	0.4270	23.6±3.5	23.7±3.2	0.5510
Smoking (%)	14.6	16.6	0.3191	12.8	12.7	0.9712
Hypertension (%)	60.2	34.7	<0.0001	86.8	52.6	<0.0001
Diabetes mellitus (%)	29.7	15.0	<0.0001	56.6	27.8	<0.0001

Data for age and BMI are means ± SD.

Table IX. Multivariable logistic regression analysis of polymorphisms related to ACI in individuals with hypercholesterolemia.

Gene	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
ENG	C→G (Asp366His)	0.7932		0.1058		0.7824		0.7954	
AGTR2	1675G→A	0.0128	0.61 (0.41-0.90)	0.0388	0.69 (0.48-0.98)	0.1502		0.0115	0.59 (0.39-0.88)
CCL5	-28C→G	0.0138	1.60 (1.10-2.32)	0.0285	3.22 (1.13-9.42)	0.0505		0.0178	3.57 (1.25-10.51)
AGTR2	3123C→A	0.0667		0.0312	1.55 (1.04-2.31)	0.6131		0.0272	1.59 (1.05-2.40)
IL6	-572G→C	0.9547		0.0051	1.66 (1.17-2.36)	0.4095		0.5815	
FABP2	2445G→A (Ala54Thr)	0.0321	1.46 (1.04-2.08)	0.0166	1.73 (1.10-2.70)	0.1697		0.0063	1.98 (1.21-3.22)
HNF4A	A→G	0.6508		0.0253	1.50 (1.05-2.14)	0.1949		0.9900	
IPF1	-108/3G→4G	0.0054	0.57 (0.38-0.85)	0.6411		0.0057	0.55 (0.36-0.84)	0.0408	0.61 (0.37-0.98)
CCL5	-403G→A	0.0634		**0.0011**	2.15 (1.36-3.39)	0.4078		0.0009	2.35 (1.42-3.89)
ADRB2	46A→G (Arg16Gly)	0.9249		0.0051	1.71 (1.17-2.50)	0.3534		0.0935	
RECQL2	T→C (Cys1367Arg)	0.1417		0.7649		0.0712		0.7670	
F7	11,496G→A (Arg353Gln)	0.1143		0.7775		0.1967		0.7764	
ACDC	G→T in intron 2	0.0211	0.67 (0.47-0.94)	0.7404		0.0116	0.63 (0.44-0.90)	0.7245	
COMT	G→A (Val158Met)	**0.0012**	0.57 (0.40-0.80)	0.3936		**0.0018**	0.56 (0.39-0.80)	0.0724	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, and diabetes mellitus. P values of <0.005 are shown in bold.

were greater, whereas the prevalence of hypercholesterolemia was lower, in subjects with ACI than in controls. Among hypertensive individuals, age and the prevalence of diabetes mellitus were greater in subjects with ACI than in controls. The Chi-square test revealed that five and 10 polymorphisms were related to ACI in normotensive or hypertensive individuals, respectively (Supplementary Table II). Multivariable logistic regression analysis with adjustment for age, sex, BMI, and the prevalence of smoking, hypercholesterolemia, and diabetes mellitus revealed that no polymorphism was associated with ACI among normotensive individuals (Supplementary Table III). Among hypertensive individuals, the -572G→C polymorphism of *IL6* (recessive model) was significantly associated with ACI (Table VI). A stepwise forward selection procedure revealed that, in descending order of statistical significance, age, sex, and smoking status significantly affected the prevalence of ACI in normotensive individuals (Table VII). For hypertensive individuals, diabetes mellitus and IL6 genotype (recessive model) significantly affected the prevalence of ACI (Table VII).

Association of polymorphisms with ACI in the absence or presence of hypercholesterolemia. Characteristics of subjects with or without hypercholesterolemia are shown in Table VIII. Among individuals with or without hypercholesterolemia, age and the prevalence of hypertension and diabetes mellitus were greater in subjects with ACI than in controls. The Chi-square test revealed that 12 and 14 polymorphisms were related to ACI in the absence or presence of hypercholesterolemia, respectively (Supplementary Table IV). Multivariable

Table X. Effects of genotypes and other characteristics on ACI in the absence or presence of hypercholesterolemia as determined by a stepwise forward selection procedure.

Hypercholesterolemia (-)			Hypercholesterolemia (+)		
Variable	P	R^2	Variable	P	R^2
Hypertension	<0.0001	0.0433	Hypertension	<0.0001	0.0931
Age	<0.0001	0.0253	Diabetes mellitus	<0.0001	0.0332
Sex	<0.0001	0.0164	*AGTR2* (*GG* versus *AA+GA*)	0.0016	0.0106
AGT (*GG* versus *AA+GA*)	0.0027	0.0045	*CCL5* (*GG +GA* versus *AA*)	0.0020	0.0101
Diabetes mellitus	0.0041	0.0041	*ADRB2* (*AA+AG* versus *GG*)	0.0036	0.0090
PCSK9 (*AA* versus *GG+AG*)	0.0086	0.0035	*COMT* (*GG* versus *AA+GA*)	0.0037	0.0089
F7 (*GG+GA* versus *AA*)	0.0095	0.0034	*IPF1* (*3G3G* versus *4G4G+3G4G*)	0.0051	0.0083
MTHFR (*CC+CT* versus *TT*)	0.0109	0.0032	*HNF4A* (*AA+AG* versus *GG*)	0.0077	0.0075
ITGB2 (*CC* versus *TT+CT*)	0.0126	0.0031	*IL6* (*GG+GC* versus *CC*)	0.0081	0.0074
Smoking	0.0449	0.0020	Sex	0.0177	0.0060
			ACDC (*GG* versus *TT+GT*)	0.0397	0.0045
			Age	0.0418	0.0044

R^2, contribution rate.

Table XI. Characteristics of subjects with ACI and controls according to the absence or presence of diabetes mellitus.

	Diabetes mellitus (-)			Diabetes mellitus (+)		
Characteristic	ACI	Controls	P	ACI	Controls	P
No. of subjects	388	1684		248	385	
Age (years)	66.7±12.1	61.6±12.1	<0.0001	68.1±9.3	65.6±11.2	0.0030
BMI (kg/m^2)	23.3±2.9	23.4±2.9	0.3450	23.4±3.4	23.6±3.6	0.6200
Smoking (%)	13.1	14.9	0.3862	15.3	18.4	0.3075
Hypertension (%)	59.8	34.1	<0.0001	84.3	64.9	<0.0001
Hypercholesterolemia (%)	24.5	25.4	0.7207	50.0	42.6	0.0680

Data for age and BMI are means ± SD.

logistic regression analysis with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, and diabetes mellitus revealed that no polymorphism was associated with ACI among individuals without hypercholesterolemia (Supplementary Table V). Among individuals with hypercholesterolemia, the -403G→A polymorphism of *CCL5* (recessive and additive 2 models) and the G→A polymorphism of *COMT* (dominant and additive 1 models) were significantly associated with ACI (Table IX). A stepwise forward selection procedure revealed that hypertension, age, sex, *AGT* genotype (dominant model), and diabetes mellitus significantly influenced ACI in individuals without hypercholesterolemia, whereas hypertension, diabetes mellitus, *AGTR2* genotype (dominant model), *CCL5* genotype (recessive model), *ADRB2* genotype (recessive model), and *COMT* genotype (dominant model) significantly affected ACI in individuals with hypercholesterolemia (Table X).

Association of polymorphisms with ACI in the absence or presence of diabetes mellitus. Characteristics of subjects with or without diabetes mellitus are shown in Table XI. Age and the prevalence of hypertension were greater in subjects with ACI than in controls for both nondiabetic and diabetic individuals. The Chi-square test revealed that eight and 14 polymorphisms were related to ACI in nondiabetic and diabetic individuals, respectively (Supplementary Table VI). Multivariable logistic regression analysis with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, and hypercholesterolemia revealed that no polymorphism was associated with ACI in nondiabetic individuals (Supplementary Table VII). In diabetic individuals, the 3932T→C polymorphism of *APOE* (dominant and additive 1 models), the 2445G→A polymorphism of *FABP2* (dominant model), and the A→G polymorphism of *TNFSF4* (additive 2 model) were significantly associated with ACI (Table XII). A stepwise forward selection procedure revealed

Table XII. Multivariable logistic regression analysis of polymorphisms related to ACI in diabetic individuals.

		Dominant		Recessive		Additive 1		Additive 2	
Gene	Polymorphism	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
APOE	3932T→C (Cys112Arg)	**0.0017**	1.98 (1.29-3.03)	0.9025		**0.0014**	2.03 (1.32-3.16)	0.8137	
FABP2	2445G→A (Ala54Thr)	**0.0038**	1.65 (1.78-2.33)	0.0692		0.0160	1.57 (1.09-2.26)	0.0107	1.94 (1.17-3.32)
TNFSF4	A→G	0.1462		0.0053	9.36 (2.33-63.04)	0.5350		**0.0048**	9.61 (2.38-64.85)
APOE	4070C→T (Arg158Cys)	0.0812				0.0812			
ABCC8	G→A (Arg1273Arg)	0.7670		0.7244		0.4686		0.7255	
GCK	-30G→A	0.7928		0.0340	0.26 (0.06-0.79)	0.3520		0.0422	0.27 (0.06-0.84)
COMT	G→A (Val158Met)	0.0063	0.62 (0.44-0.87)	0.4109		0.0094	0.62 (0.35-1.17)	0.1117	
MTHFR	677C→T (Ala222Val)	0.5767		0.0250	1.66 (1.07-2.58)	0.1624		0.1571	
APOH	341G→A (Ser88Asn)	0.7588				0.7588			
ENG	C→G (Asp366His)	0.7674		0.5544		0.7609		0.7684	
P2RY12	744T→C	0.9633		0.0384	0.31 (0.09-0.86)	0.4371		0.0462	0.32 (0.09-0.89)
GP1BA	1018C→T (Thr145Met)	0.6972		0.7416		0.9659		0.7415	
GYS1	A→G (Met416Val)	0.0132	0.57 (0.37-0.88)	0.5491		0.0076	0.54 (0.34-0.84)	0.6608	
MMP2	-1306C→T	0.0563		0.7812		0.0321	0.59 (0.36-0.95)	0.7841	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, and hypercholesterolemia. P values of <0.005 are shown in bold.

Table XIII. Effects of genotypes and other characteristics on ACI in the absence or presence of diabetes mellitus as determined by a stepwise forward selection procedure.

Diabetes mellitus (-)			Diabetes mellitus (+)		
Variable	P	R^2	Variable	P	R^2
Hypertension	<0.0001	0.0428	Hypertension	<0.0001	0.0352
Age	<0.0001	0.0170	*APOE* (*TT* versus *CC+TC*)	0.0015	0.0118
Sex	<0.0001	0.0140	Age	0.0017	0.0116
IL6 (*GG+GC* versus *CC*)	0.0049	0.0040	*TNFSF4* (*AA+AG* versus *GG*)	0.0019	0.0114
MMP12 (*AA* versus *GG+AG*)	0.0083	0.0035	*GCK* (*GG+GA* versus *AA*)	0.0038	0.0099
COL3A1 (*GG+GA* versus *AA*)	0.0113	0.0032	Sex	0.0045	0.0095
SAH (*AA* versus *GG+AG*)	0.0139	0.0030	*FABP2* (*GG* versus *AA+GA*)	0.0092	0.0080
ABCA1 (*AA+AG* versus *GG*)	0.0237	0.0026	*COMT* (*GG* versus *AA+GA*)	0.0115	0.0075
Smoking	0.0290	0.0024	*MTHFR* (*CC+CT* versus *TT*)	0.0179	0.0066
			P2RY12 (*TT+TC* versus *CC*)	0.0250	0.0059
			Hypercholesterolemia	0.0326	0.0054
			GYS1 (*AA* versus *GG+AG*)	0.0420	0.0049

R^2, contribution rate.

that hypertension, age, sex, and IL6 genotype (recessive model) significantly affected ACI in nondiabetic individuals (Table XIII). Hypertension, *APOE* genotype (dominant model), age, *TNFSF4* genotype (recessive model), *GCK* genotype (recessive model), and sex significantly influenced ACI in diabetic individuals (Table XIII).

The polymorphisms significantly (P<0.005) associated with ACI by both multivariable logistic regression analysis and the stepwise forward selection procedure in women or men, or in individuals with or without hypertension, diabetes mellitus, or hypercholesterolemia, are summarized in Table XIV.

Discussion

We have examined the relation of 202 polymorphisms to ACI in women or men separately as well as in individuals with or

Table XIV. Summary of polymorphisms significantly (P<0.005) associated with ACI as determined by multivariable logistic regression analysis and a stepwise forward selection procedure.

Sex or risk factor	Gene	Polymorphism	Risk allele	Function of encoded protein
Women	*LIPG*	584C→T (Thr111Ile)	*C*	Phospholipase involved in HDL and apolipoprotein A-1 metabolism
	EDN1	5665G→T (Lys198Asn)	*G*	Vasoconstrictor produced by vascular endothelial cells
	CCL11	G→A (Ala23Thr)	*G*	Chemokine involved in allergic inflammation and angiogenesis
Men	*MTHFR*	677C→T (Ala222Val)	*T*	Enzyme involved in methylation of homocysteine
	ITGB2	1323C→T	*C*	Integrin that participates in leukocyte adhesion and cellular signaling
	APOE	3932T→C (Cys112Arg)	*T*	Ligand for the LDL receptor and the LDL receptor-related protein
	EDNRA	-231A→G	*G*	Endothelin 1 receptor that modulates vascular tone
Hypertension (-) Hypertension (+)	*IL6*	-572G→C	*C*	Cytokine involved in acute inflammatory responses
Hypercholesterolemia (-) Hypercholesterolemia (+)	*CCL5*	-403G→A	*A*	Chemoattractant for monocytes, T helper cells, and eosinophils
	COMT	G→A (Val158Met)	*G*	Enzyme that catalyzes catecholamine transmitters
Diabetes mellitus (-) Diabetes mellitus (+)	*APOE*	3932T→C (Cys112Arg)	*T*	See above
	TNFSF4	A→G	*G*	Ligand that mediates adhesion of activated T cells to endothelial cells

HDL, high density lipoprotein; LDL, low density lipoprotein.

without hypertension, hypercholesterolemia, or diabetes mellitus. Our observations suggest that polymorphisms associated with ACI may differ between men and women as well as among individuals with or without different conventional risk factors for atherosclerosis.

ACI is the most common type of stroke and, in most patients, is caused by atherosclerosis (5). Atherosclerosis results from excessive inflammatory and fibroproliferative responses to various forms of insult to the endothelium and smooth muscle of the artery wall, with the participation of large numbers of growth factors, cytokines, and vasoregulatory molecules (17). We therefore selected 152 candidate genes for ACI on the basis of a comprehensive overview of vascular, platelet, leukocyte, lymphocyte, and monocyte-macrophage biology; coagulation and fibrinolysis cascades; neurological factors; as well as lipid, glucose, and homocysteine metabolism and other metabolic factors. Indeed, the genes found to be associated with ACI may have roles in diverse aspects of the etiology of this condition, including cell adhesion (*ITGB2*, *TNFSF4*); vascular inflammation (*CCL11, IL6*); leukocyte, lymphocyte, and monocyte-macrophage biology (*CCL5*); vascular constriction (*EDN1, EDNRA*); and metabolism of lipids (*LIPG, APOE*), homocysteine (*MTHFR*), and catecholamine transmitters (*COMT*). Three of the 11 polymorphisms associated with ACI in the present study (677C→T of *MTHFR*, 3932T→C of *APOE*, and -572G→C of *IL6*) have previously been associated with ischemic stroke (8,9,15,18,19). The remaining eight polymorphisms (584C→T of *LIPG*, 5665G→T of *EDN1*, G→A of *CCL11*, 1323C→T of *ITGB2*, -231A→G of *EDNRA*, -403G→A of *CCL5*, G→A of *COMT*, and A→G of *TNFSF4*) have not been previously associated with this condition.

Association of polymorphisms with ACI in women versus men. The 584C→T polymorphism of *LIPG*, the 5665G→T polymorphism of *EDN1*, and the G→A polymorphism of *CCL11* were associated with ACI in women, whereas the 677C→T polymorphism of *MTHFR*, the 1323C→T polymorphism of ITGB2, the 3932T→C polymorphism of *APOE*, and the -231A→G polymorphism of *EDNRA* were associated with this condition in men. The mechanisms responsible for the difference in the polymorphisms associated with ACI between men and women remain unclear. Given that, in general, the total risk for atherosclerotic disease, such as coronary heart disease and ACI, in women lags behind that in men by approximately 10 years, the mechanisms underlying the risk for ACI in women may differ from those in men at each age. The sex difference in the association of poly-

morphisms with ACI might be attributable to the difference in sex hormones such as estrogen between men and women, given that estrogen exerts various favorable effects on vessel wall and vasomotor function, including stimulation of the production of nitric oxide and prostaglandin I_2 as well as inhibition of the release of endothelin-1 by vascular endothelial cells (20). Furthermore, considering that the polymorphisms examined in our study probably represent only a small proportion of those potentially associated with ACI, it remains possible that further investigations will uncover polymorphisms that are associated with ACI in both men and women.

Association of polymorphisms with ACI in the absence or presence of conventional risk factors for atherosclerosis. Given that interactions between gene polymorphisms and conventional risk factors may be important in the etiology of ACI, we examined the effects of polymorphisms on the prevalence of ACI in the absence or presence of hypertension, hypercholesterolemia, or diabetes mellitus. The -572G→C polymorphism of *IL6* was associated with ACI in hypertensive individuals, whereas no polymorphism was associated with ACI in normotensive individuals. The -403G→A polymorphism of *CCL5* and the G→A polymorphism of *COMT* were associated with ACI in subjects with hypercholesterolemia, whereas no polymorphism was associated with ACI in subjects without hypercholesterolemia. The 3932T→C polymorphism of *APOE* and the A→G polymorphism of *TNFSF4* were associated with ACI in diabetic individuals, whereas no polymorphism was associated with this condition in nondiabetic subjects. These observations suggest that polymorphisms associated with ACI may differ among subjects with different conventional risk factors, although the mechanisms responsible for these differences remain to be elucidated. Given that the effects of single polymorphisms on the development of ACI are likely to be small, the association between a polymorphism and the prevalence of ACI might be influenced by the absence or presence of conventional risk factors for atherosclerosis. Furthermore, considering that hypertension, hypercholesterolemia, and diabetes mellitus probably have genetic components, there might be interactions between genes related to ACI and those related to conventional risk factors.

There were several limitations to the present study. The number of subjects with ACI was relatively small after stratification of these individuals by sex or conventional risk factors. Considering the multiple comparisons of genotypes with ACI, we adopted the criterion of $P<0.005$ for significant association. However, this approach does not completely exclude the possibility of false positive associations. It is also possible that one or more of the polymorphisms associated with ACI in our study are in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for the development of this condition. Furthermore, the functional relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study.

Our present observations suggest that polymorphisms associated with ACI may differ between women and men as well as among individuals with different conventional risk factors for atherosclerosis. Stratification of subjects on the basis of sex or conventional risk factors may thus be important in order to achieve the personalized prevention of ACI with the use of genetic information.

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Supplementary Table I. Polymorphisms related to ACI in women or men as determined by the Chi-square test.

Women			Men		
Gene	Polymorphism	P	Gene	Polymorphism	P
LIPG	584C→T (Thr111Ile)	0.0007	*MTHFR*	677C→T (Ala222Val)	0.0011
CCL11	G→A (Ala23Thr)	0.0010	*ITGB2*	1323C→T	0.0031
EDN1	5665G→T (Lys198Asn)	0.0027	*THBS2*	T→G (3' UTR)	0.0050
AKAP10	A→G (Ile646Val)	0.0044	*ALOX5*	G→A (Glu254Lys)	0.0083
UTS2	G→A (Ser89Asn)	0.0120	*IPF1*	-108/3G→4G	0.0220
IL6	-572G→C	0.0186	*APOE*	3932T→C (Cys112Arg)	0.0284
PTGDS	4111A→C	0.0195	*APOE*	-219G→T	0.0320
ANXA5	-1C→T	0.0197	*KCNJ11*	A→G (Glu23Lys)	0.0327
KCNJ11	A→G (Glu23Lys)	0.0210	*ACDC*	-11,377C→G	0.0327
			APOA1	84T→C	0.0355
			APOH	341G→A (Ser88Asn)	0.0355
			SELE	561A→C (Ser128Arg)	0.0358
			TNFSF4	A→G	0.0369
			F7	11,496G→A (Arg353Gln)	0.0387
			UCP3	-55C→T	0.0468
			EDNRA	-231A→G	0.0468

Supplementary Table II. Polymorphisms related to ACI in the absence or presence of hypertension as determined by the Chi-square test.

Hypertension (-)			Hypertension (+)		
Gene	Polymorphism	P	Gene	Polymorphism	P
MTHFR	677C→T (Ala222Val)	0.0070	*IL6*	-572G→C	0.0067
NOS3	-786 T→C	0.0241	*SLC26A8*	A→G (Ile639Val)	0.0149
SAH	A→G in intron 12	0.0418	*GP6*	13,254T→C (Ser219Pro)	0.0268
UCP3	-55C→T	0.0473	*THBS2*	T→G (3' UTR)	0.0316
AGTR1	1166A→C	0.0497	*TNFSF4*	A→G	0.0329
			PCSK9	23,968A→G (Glu670Gly)	0.0329
			IPF1	-108/3G→4G	0.0362
			CCR5	59,029G→A	0.0367
			SELE	561A→C (Ser128Arg)	0.0404
			HNF4A	A→G	0.0465

Supplementary Table III. Multivariable logistic regression analysis of polymorphisms related to ACI in normotensive individuals.

		Dominant		Recessive		Additive 1		Additive 2	
Gene	Polymorphism	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
MTHFR	677C→T (Ala222Val)	0.9203		0.0073	1.69 (1.14-2.47)	0.3760		0.0495	1.54 (1.00-2.38)
NOS3	-786T→C	0.0731		0.7701		0.1461		0.7688	
SAH	A→G in intron 12	0.0323	0.32 (0.10-0.81)	0.8734		0.0334	0.32 (0.10-0.81)	0.8721	
UCP3	-55C→T	0.4095		0.0964		0.1473		0.2580	
AGTR1	1166A→C	0.2329		0.0095	4.96 (1.39-16.53)	0.5319		0.0087	5.08 (1.42-16.95)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, BMI, and the prevalence of smoking, hypercholesterolemia, and diabetes mellitus.

Supplementary Table IV. Polymorphisms related to ACI in the absence or presence of hypercholesterolemia as determined by the Chi-square test.

Hypercholesterolemia (-)			Hypercholesterolemia (+)		
Gene	Polymorphism	P	Gene	Polymorphism	P
F7	11,496G→A (Arg353Gln)	0.0057	*ENG*	C→G (Asp366His)	0.0003
AGT	-6G→A	0.0076	*AGTR2*	1675G→A	0.0037
MTHFR	677C→T (Ala222Val)	0.0088	*CCL5*	-28C→G	0.0046
ITGB2	1323C→T	0.0095	*AGTR2*	3123C→A	0.0058
UTS2	G→A (Ser89Asn)	0.0128	*IL6*	-572G→C	0.0076
ANXA5	-1C→T	0.0143	*FABP2*	2445G→A (Ala54Thr)	0.0133
SELE	561A→C (Ser128Arg)	0.0214	*HNF4A*	A→G	0.0228
LIPG	584C→T (Thr111Ile)	0.0252	*IPF1*	-108/3G→4G	0.0292
UCP3	-55C→T	0.0374	*CCL5*	-403G→A	0.0300
IL6	-572G→C	0.0382	*ADRB2*	46A→G (Arg16Gly)	0.0375
PAI1	A→G (Tyr243Cys)	0.0459	*RECQL2*	T→C (Cys1367Arg)	0.0376
PCSK9	23,968A→G (Glu670Gly)	0.0467	*F7*	11,496G→A (Arg353Gln)	0.0379
			ACDC	G→T in intron 2	0.0425
			COMT	G→A (Val158Met)	0.0431

Supplementary Table V. Multivariable logistic regression analysis of polymorphisms related to ACI in individuals without hypercholesterolemia.

		Dominant		Recessive		Additive 1		Additive 2	
Gene	Polymorphism	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
F7	11,496G→A (Arg353Gln)	0.9484		0.0222	7.03 (1.50-50.38)	0.6667		0.0229	6.97 (1.48-49.92)
AGT	-6G→A	0.0124	3.01 (1.37-7.95)	0.4913		0.0063	3.41 (1.52-9.12)	0.0183	2.84 (1.29-7.53)
MTHFR	677C→T (Ala222Val)	0.7596		0.0115	1.46 (1.09-1.96)	0.2160		0.0817	
ITGB2	1323C→T	0.0146	1.33 (1.06-1.67)	0.6545		0.0082	1.38 (1.09-1.74)	0.9249	
UTS2	G→A (Ser89Asn)	0.2625		0.0496	0.51 (0.25-0.96)	0.0903		0.0861	
ANXA5	-1C→T	0.4968		0.6264		0.7900		0.6261	
SELE	561A→C (Ser128Arg)	0.1094		0.1456		0.1880		0.1413	
LIPG	584C→T (Thr111Ile)	0.0414	1.27 (1.01-1.60)	0.0986		0.0961		0.0501	
UCP3	-55C→T	0.5128		0.1079		0.2105		0.2496	
IL6	-572G→C	0.0913		0.0813		0.1941		0.0624	
PAI1	A→G (Tyr243Cys)	0.7340				0.7340			
PCSK9	23,968A→G (Glu670Gly)	0.0087	1.64 (1.13-2.36)	0.8706		0.0064	1.69 (1.15-2.45)	0.9131	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, and diabetes mellitus.

Supplementary Table VI. Polymorphisms related to ACI in the absence or presence of diabetes mellitus as determined by the Chi-square test.

Diabetes mellitus (-)			Diabetes mellitus (+)		
Gene	Polymorphism	P	Gene	Polymorphism	P
MMP12	-82 A→G	0.0040	*APOE*	3932T→C (Cys112Arg)	0.0030
IL6	-572 G→C	0.0118	*FABP2*	2445G→A (Ala54Thr)	0.0035
SELE	561A→C (Ser128Arg)	0.0133	*TNFSF4*	A→G	0.0043
SAH	A→G in intron 12	0.0151	*APOE*	4070C→T (Arg158Cys)	0.0196
ANXA5	-1C→T	0.0174	*ABCC8*	G→A (Arg1273Arg)	0.0211
COL3A1	A→G (Ile1205Val)	0.0265	*GCK*	-30G→A	0.0212
ABCA1	2583A→G (Ile823Met)	0.0294	*COMT*	G→A (Val158Met)	0.0237
COL3A1	G→A (Ala698Thr)	0.0474	*MTHFR*	677C→T (Ala222Val)	0.0239
			APOH	341G→A (Ser88Asn)	0.0254
			ENG	C→G (Asp366His)	0.0271
			P2RY12	744T→C	0.0377
			GP1BA	1018C→T (Thr145Met)	0.0415
			GYS1	A→G (Met416Val)	0.0422
			MMP2	-1306C→T	0.0466

Supplementary Table VII. Multivariable logistic regression analysis of polymorphisms related to ACI in nondiabetic individuals.

		Dominant		Recessive		Additive 1		Additive 2	
Gene	Polymorphism	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
MMP12	-82A→G	0.0052	2.05 (1.22-3.36)			0.0052	2.05 (1.22-3.36)		
IL6	-572G→C	0.0854		0.0066	1.40 (1.10-1.78)	0.2962		0.0412	1.86 (1.06-3.51)
SELE	561A→C (Ser128Arg)	0.0393	1.57 (1.01-2.38)	0.1465		0.0760		0.1415	
SAH	A→G in intron 12	0.0364	0.43 (0.18-0.89)	0.8618		0.0380	0.43 (0.18-0.89)	0.8607	
ANXA5	-1C→T	0.3737		0.7237		0.6536		0.7233	
COL3A1	A→G (Ile1205Val)	0.1596		0.6908		0.2372		0.6894	
ABCA1	2583A→G (Ile823Met)	0.4747		0.0256	1.31 (1.04-1.66)	0.1069		0.6835	
COL3A1	G→A (Ala698Thr)	0.1379		0.0108	1.65 (1.11-2.40)	0.4701		0.0081	1.71 (1.14-2.53)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, BMI, and the prevalence of smoking, hypertension, and hypercholesterolemia.