Association of genetic variants with atherothrombotic cerebral infarction in Japanese individuals with metabolic syndrome

YOSHIJI YAMADA[1], KIMIHIKO KATO[2], MITSUTOSHI OGURI[2], TETSURO YOSHIDA[2], KIYOSHI YOKOI[2], SACHIRO WATANABE[3], NORIFUMI METOKI[4], HIDEMI YOSHIDA[5], KEI SATOH[5], SAHOKO ICHIHARA[1], YUKITOSHI AOYAGI[6], AKITOMO YASUNAGA[6], HYUNTAE PARK[6], MASASHI TANAKA[6] and YOSHINORI NOZAWA[7]

[1]Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu; [2]Department of Cardiovascular Medicine, Gifu Prefectural Tajimi Hospital, Tajimi; [3]Department of Cardiology, Gifu Prefectural General Medical Center, Gifu; [4]Department of Internal Medicine, Hirosaki Stroke Center, Hirosaki; [5]Department of Vascular Biology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki; [6]Department of Genomics for Longevity and Health, Tokyo Metropolitan Institute of Gerontology, Tokyo; [7]Gifu International Institute of Biotechnology, Kakamigahara, Japan

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Abstract. Metabolic syndrome is a risk factor for cardiovascular disease. The aim of the present study was to identify genetic variants that confer susceptibility to atherothrombotic cerebral infarction among individuals with metabolic syndrome in order to allow prediction of genetic risk for this condition. The study population comprised 1284 unrelated Japanese individuals with metabolic syndrome, including 313 subjects with atherothrombotic cerebral infarction and 971 controls. The genotypes for 296 polymorphisms of 202 candidate genes were determined with a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. The Chi-square test, multivariable logistic regression analysis with adjustment for age, sex, body mass index, and the prevalence of hypertension, hypercholesterolemia, and diabetes mellitus, as well as a stepwise forward selection procedure revealed that the 2445G→A (Ala54Thr) polymorphism (rs1799883) of *FABP2*, the -108/3G→4G polymorphism of *IPF1* (S82168), the A→G (Thr94Ala) polymorphism (rs2241883) of *FABP1*, the G→A (Asp2213Asn) polymorphism (rs529038) of *ROS1*, the -11377C→G polymorphism (rs266729) of *ADIPOQ*, the 162A→C polymorphism (rs4769055) of *ALOX5AP*, the -786T→C polymorphism (rs2070744) of *NOS3*, and the 3279C→T polymorphism (rs7291467) of *LGALS2* were associated ($P<0.05$) with the prevalence of atherothrombotic cerebral infarction. Among these polymorphisms, the 2445G→A (Ala54Thr) polymorphism of *FABP2* was most significantly associated with this condition. Our results suggest that *FABP2*, *IPF1*, *FABP1*, *ROS1*, *ADIPOQ*, *ALOX5AP*, *NOS3*, and *LGALS2* are susceptibility loci for atherothrombotic cerebral infarction among Japanese individuals with metabolic syndrome. Genotypes for these polymorphisms, especially for the 2445G→A (Ala54Thr) polymorphism of *FABP2*, may prove informative for the prediction of genetic risk for atherothrombotic cerebral infarction among such individuals.

Correspondence to: Dr Yoshiji Yamada, FAHA, 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

Metabolic syndrome is defined by a clustering of abdominal obesity, an increased serum concentration of triglycerides, a decreased serum concentration of high density lipoprotein (HDL)-cholesterol, high blood pressure, and an increased fasting blood glucose level (1). Although metabolic syndrome has been recognized as a risk factor for atherosclerotic diseases such as coronary heart disease (2,3) and ischemic stroke (4-8), genetic risk for ischemic stroke in individuals with metabolic syndrome has remained uncharacterized. Given that stroke is the leading cause of severe disability and the third leading cause of death, after heart disease and cancer, in western countries and Japan (9), the identification of biomarkers for stroke risk is important both for risk prediction and for intervention to avert future events.

In light of the above, we performed an association study for 296 candidate gene polymorphisms and atherothrombotic cerebral infarction in 1284 Japanese individuals with metabolic syndrome. The aim of the present study was to identify genetic variants that confer susceptibility to atherothrombotic

cerebral infarction among individuals with metabolic syndrome in order to allow prediction of genetic risk for this condition.

Materials and methods

Study population. The study population comprised 1284 unrelated Japanese individuals who visited outpatient clinics of, or were admitted to, one of the participating hospitals (Gifu Prefectural General Medical Center and Gifu Prefectural Tajimi Hospital in Gifu Prefecture, Japan; and Hirosaki University Hospital, Reimeikyo Rehabilitation Hospital, and Hirosaki Stroke Center in Aomori Prefecture, Japan) between October 2002 and June 2007 because of various symptoms or for an annual health checkup, or who were recruited to a population-based prospective cohort study of aging and age-related diseases in Gunma Prefecture, Japan. Diagnosis of metabolic syndrome was based on a modified version of the definition of metabolic syndrome proposed by the American Heart Association and the US National Heart, Lung, and Blood Institute (1). In this modified version, which was also used in the West of Scotland Coronary Prevention Study (10) and the Women's Health Study (11), body mass index (BMI) replaces waist circumference. On the basis of the recent recognition of a need to revise BMI criteria for obesity in Japanese and other Asian populations (12), we set the cutoff point for obesity as a BMI of ≥25 kg/m^2. A total of 1284 subjects with metabolic syndrome had thus three or more of the following five components: i) a BMI of ≥25 kg/m^2; ii) a serum concentration of triglycerides of ≥1.65 mmol/l (150 mg/dl) or drug treatment for elevated triglycerides; iii) a serum concentration of HDL-cholesterol of <1.04 mmol/l (40 mg/dl) for men or <1.30 mmol/l (50 mg/dl) for women, or drug treatment for reduced HDL-cholesterol; iv) a systolic blood pressure of ≥130 mmHg or diastolic blood pressure of ≥85 mmHg, or drug treatment for hypertension; and v) a fasting plasma glucose level of ≥5.50 mmol/l (100 mg/dl) or drug treatment for elevated glucose.

Among the 1284 subjects with metabolic syndrome, 313 individuals (193 men, 120 women) had atherothrombotic cerebral infarction. 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 >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 (13). Individuals with cardiogenic embolic infarction, lacunar infarction alone, transient ischemic attack, moyamoya disease, or cerebral venous sinus thrombosis were excluded from the study, as were those with atrial fibrillation in the absence or presence of valvular heart disease. The 971 control subjects (473 men, 498 women) had metabolic syndrome but 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. The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine, Hirosaki University

Table I. Primers, probes, and other PCR conditions for genotyping of polymorphisms examined in the study.

Gene	Polymorphism	Sense primer	Antisense primer	Probe 1	Probe 2	AT	Cy
NOS3	-786T→C	CCACCTGCATTCTGGGAACTG	CTGTCATTCAGTGACGCACGCT	CAGGGTCAGCCAGCCAGGGA	CTCTTCCCTGGCCGGCTGAC	60	50
FABP2	2445G→A (Ala54Thr)	AGCTGACAATTACACAAGAAGGAA	GTTGTAATTAAAGGTGACACCAAG	AATGTTTCGAAAAGCGCTTGATT	TCAAAGAATCAAGCACTTTTCGA	60	50
ADRB3	190T→C (Trp64Arg)	GGGAGGCAACCTGCTGGTCAT	GCTGCGGCCAGCGAAGTCA	GTCTCGGAGTCCAGGCGAT	TCTCGGAGTCCGGGCGAT	60	50
ALOX5AP	162A→C	AGGCAATGTTGTCCTGTTGGCCATCG	GCCTGACTTCCAAACAACCATCAAAG	AAGGAAAGCCCTTCAATCAGG	CTTCCCTGAGTGAAGGGC	60	50
HMOX1	-413T→A	GGGGTTGCTAAGTTCCTGATGT	GGCGTCCCAGAAGGTTCCAG	CCACCAGGCTATTGCTCTGA	TGCTCAGAGCAAAAGCCTGGT	60	50
FABP1	A→G (Thr94Ala)	TCTCTGTTCCCTGCAGACAGTGG	GTCGCCGTTGAGTTCGGTCA	AACTGGTGACAACTTTCAA	AACTGtTGACAGCTTTCAAAAACA	60	50
THBS2	3949T→G	AACCCAAGTGCCTTCAGAGGAT	CTCCACATAAAGTCTCATATATCAC	GATGTTCATCTCTGAGTTCCA	GATGTTCATCTCTGCGTTCCA	60	50
LTA4H	A→G (rs2660845)	CTTCCTGTGGACTTCATAGTGTCTACC	CTGACGCAGGGTGTATCGAGCC	CTACCACTGGCCCCACGGTGCT	AAGCTGCAGAGCCCCGCGGGTCCA	60	50
LGALS2	3279C→T	AGGGAGCCATCTCCTGATGCT	GCCACACAGACACTCACAGAC	CGCACACACACGTCTAACA	CGCACACACATCTCTAACAC	60	50
LIPC	-250G→A	CAGCCACGTGGAAGCCACCT	TCGATTTACAGAAGTGCTTCTTATC	CCAAATTAATCAATTTAAAGCTACT	GTTCCAAATTAATCAACTTAAAGCT	60	50
ADIPOQ	-11377C→G	TAATTCATCAGAATGTGTGGCTTG	TTAGGCTTGAAGTGGCAACATTC	GCTCAGATCCTGCCCTTCAAA	GTTTTGTTTTTGAAGCGCAGGAT	60	50
LTA4H	A→G (rs2540482)	TTATAATATACTGTGAATAACTGGTTA	CCTTCAAGGTCTTACTAACATTGCC	AAAGCTTACATTCATCTTTTAATCCCT	CAAGGGATTAAAAGATGAACGTAAGC	60	50
ADIPOR2	795G→A	CATCTGTGTGCTGGGCATTG	CCCCGTGCTCTTACCTGCTC	TAGTCTCCCAGTGGGACAT	TAGTCTCCCAATGGGACATG	60	50
IPF1	-108/3G→4G	TGGCTGTGGGTTCCCTCTGAG	GATTTGGCACTGTGTGGCGTTC	CGAGCAGGGGTGGCGCC	GGCGCCACCCTGCTCGCT	60	50
LIPC	-514C→T	TGGCAAGGCATCTTTGCTTC	TGGGTTCAGTGAAATTGGTGATGC	TTCACCCCCGTGTCAAAAGG	TTCACCCCCATGTCAAAAGG	60	50
ROS1	G→A (Asp2213Asn)	TGGGCTCAAGAACCCGACCAA	TGACTCCACTGTTGTTTGCTTCAT	AACTGAAGTTGGTCCTGAATT	AACTGAAGTTGGTTCTGAATTC	60	50
ROS1	G→C (Cys2229Ser)	TCAGAACCAACTTCAGTTATTCAGAA	AGCTTTCATTTATGACTCCACTGTTG	GCATTTATIAGTGCAGAGATGA	GCATTTATIAGTCCAGAGATGAAGC	60	50

Oligonucleotide sequences are 5'→3'. AT, annealing temperature (˚C); Cy, cycles.

Table II. Characteristics of subjects with atherothrombotic cerebral infarction (ACI) and controls among individuals with metabolic syndrome.

Characteristic	ACI	Controls	P
No. of subjects	313	971	
Age (years)	67.0±9.7	68.2±9.2	0.0508
Sex (male/female, %)	61.7/38.3	48.7/51.3	<0.0001
BMI (kg/m^2)	24.5±3.5	25.3±3.2	0.0001
Current or former smoker (%)	23.7	24.1	0.8886
Hypertension (%)	87.2	63.8	<0.0001
Systolic blood pressure (mmHg)	153±27	144±20	<0.0001
Diastolic blood pressure (mmHg)	84±17	82±12	0.0022
Hypercholesterolemia (%)	53.3	36.8	<0.0001
Serum total cholesterol (mmol/l)	5.35±1.11	5.26±0.94	0.1422
Serum triglycerides (mmol/l)	1.97±1.10	2.20±1.34	0.0057
Serum HDL-cholesterol (mmol/l)	1.18±0.35	1.26±0.32	0.0003
Diabetes mellitus (%)	57.2	25.5	<0.0001
Fasting plasma glucose (mmol/l)	7.66±2.94	7.40±3.30	0.2184
Glycosylated hemoglobin (%)	6.27±1.50	5.84±1.48	0.0003

Quantitative data are means ± SD. Smoker: smoking ≥10 cigarettes daily. Hypertension: systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg (or both), or taking antihypertensive medication. Hypercholesterolemia: serum total cholesterol of ≥5.72 mmol/l (220 mg/dl) or taking lipid-lowering medication. Diabetes mellitus: fasting blood glucose of ≥6.93 mmol/l (126 mg/dl) or glycosylated hemoglobin content (hemoglobin A1c) of ≥6.5% (or both), or taking antidiabetes medication.

Graduate School of Medicine, Gifu International Institute of Biotechnology, Tokyo Metropolitan Institute of Gerontology, and participating hospitals. Written informed consent was obtained from each participant.

Selection and genotyping of polymorphisms. Our aim was to identify genetic variants associated with atherothrombotic cerebral infarction among Japanese individuals with metabolic syndrome in a case-control association study by examining the relations of one to five polymorphisms of each candidate gene with this condition. With the use of public databases, including PubMed (NCBI) and Online Mendelian Inheritance in Man (NCBI), we selected 202 candidate genes that have been characterized and suggested to be associated with atherothrombotic cerebral infarction. On the basis of published studies or by searching PubMed and single nucleotide polymorphism (SNP) databases [dbSNP (NCBI) and Japanese SNP database (JSNP)], we further selected 296 polymorphisms of these genes, most located in the promoter region or exons, that might be expected to result in changes in the function or expression of the encoded protein (14,15). Wild-type and variant alleles of the polymorphisms were determined from the original sources.

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 296 polymorphisms were determined at G&G Science (Fukushima, Japan) by a method that combines the polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Primers, probes, and other PCR conditions for genotyping polymorphisms found to be related (P<0.05) to atherothrombotic cerebral infarction by the Chi-square test are shown in Table I. Detailed genotyping methodology was described previously (16).

Statistical analysis. Quantitative data were compared between subjects with atherothrombotic cerebral infarction and controls by the unpaired Student's t-test. Categorical 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-Weinberg equilibrium. In the initial screen, genotype distributions for each polymorphism were compared between subjects with atherothrombotic cerebral infarction and controls with the Chi-square test. Polymorphisms with a P-value of <0.05 were further examined in a more rigorous evaluation of association by multivariable logistic regression analysis with adjustment for covariates that differed significantly between subjects with atherothrombotic cerebral infarction and controls. Given that the difference in age was marginally significant, it was included in covariates. Multivariable logistic regression analysis was thus performed with atherothrombotic cerebral infarction as a dependent variable and independent variables including age, sex (0, woman; 1, man), BMI, metabolic variables (0, no history of hypertension, diabetes mellitus, or hypercholesterolemia; 1, positive history), and genotype of each polymorphism, and the P-value, odds ratio, and 95% confidence interval were calculated. Genotypes were assessed according to dominant,

Table III. Genotype distributions of polymorphisms related (P<0.05) to atherothrombotic cerebral infarction (ACI) among individuals with metabolic syndrome as determined by the Chi-square test.

Gene symbol	Polymorphism	dbSNP[a]	ACI		Controls		P
NOS3	-786T→C	rs2070744					0.0025
	TT		256	(82.3)	748	(77.0)	
	TC		46	(14.8)	213	(21.9)	
	CC		9	(2.9)	10	(1.0)	
FABP2	2445G→A (Ala54Thr)	rs1799883					0.0028
	GG		114	(36.7)	448	(46.1)	
	GA		140	(45.0)	405	(41.7)	
	AA		57	(18.3)	118	(12.2)	
ADRB3	190T→C (Trp64Arg)	rs4994					0.0104
	TT		215	(69.1)	627	(64.6)	
	TC		78	(25.1)	314	(32.3)	
	CC		18	(5.8)	30	(3.1)	
ALOX5AP	162A→C	rs4769055					0.0104
	AA		93	(29.9)	231	(23.8)	
	AC		159	(51.1)	483	(49.7)	
	CC		59	(19.0)	257	(26.5)	
HMOX1	-413T→A	rs2071746					0.0105
	TT		69	(22.2)	292	(30.1)	
	TA		167	(53.7)	438	(45.1)	
	AA		75	(24.1)	241	(24.8)	
FABP1	A→G (Thr94Ala)	rs2241883					0.0129
	AA		162	(52.1)	581	(59.9)	
	AG		137	(44.1)	338	(34.9)	
	GG		12	(3.9)	51	(5.3)	
THBS2	3949T→G (3'-UTR)	rs8089					0.0133
	TT		247	(79.4)	832	(85.7)	
	TG		60	(19.3)	136	(14.0)	
	GG		4	(1.3)	3	(0.3)	
LTA4H	A→G	rs2660845					0.0157
	AA		60	(19.3)	150	(15.5)	
	AG		125	(40.2)	480	(49.4)	
	GG		126	(40.5)	341	(35.1)	
LGALS2	3279C→T (intron 1)	rs7291467					0.0181
	CC		153	(49.2)	426	(43.9)	
	CT		137	(44.1)	429	(44.2)	
	TT		21	(6.8)	116	(12.0)	
LIPC	-250G→A	rs2070895					0.0187
	GG		91	(29.3)	246	(25.3)	
	GA		127	(40.8)	485	(50.0)	
	AA		93	(29.9)	240	(24.7)	
ADIPOQ	-11377C→G	rs266729					0.0207
	CC		163	(52.4)	575	(59.2)	
	CG		120	(38.6)	346	(35.6)	
	GG		28	(9.0)	50	(5.2)	
LTA4H	A→G	rs2540482					0.0214
	AA		58	(18.0)	145	(15.1)	
	AG		124	(39.9)	470	(48.9)	
	GG		131	(42.1)	347	(36.1)	
ADIPOR2	795G→A	rs16928751					0.0255
	GG		303	(97.4)	962	(99.2)	
	GA		8	(2.6)	8	(0.8)	
	AA		0	(0)	0	(0)	

Table III. Continued.

Gene symbol	Polymorphism	dbSNP[a]	ACI		Controls		P
IPF1	-108/3G→4G	S82168					0.0280
	3G3G		96	(30.9)	226	(23.3)	
	3G4G		134	(43.1)	475	(48.9)	
	4G4G		81	(26.1)	270	(27.8)	
LIPC	-514C→T	rs1800588					0.0296
	CC		91	(29.3)	239	(24.6)	
	CT		130	(41.8)	489	(50.4)	
	TT		90	(28.9)	242	(25.0)	
ROS1	G→A (Asp2213Asn)	rs529038					0.0311
	GG		225	(72.4)	709	(73.0)	
	GA		74	(23.8)	249	(25.6)	
	AA		12	(3.9)	13	(1.3)	
ROS1	G→C (Cys2229Ser)	rs619203					0.0375
	GG		10	(4.1)	12	(1.3)	
	GC		50	(20.3)	196	(21.5)	
	CC		186	(75.6)	702	(77.1)	

Numbers in parentheses are percentages. [a]In instances in which rs numbers in dbSNP were not detected, NCBI GenBank accession numbers are shown.

recessive, and two additive (additive 1 and 2) genetic models. Each genetic model comprised two groups: the combined group of variant homozygotes and heterozygotes versus wild-type homozygotes for the dominant model; variant homozygotes versus the combined group of wild-type homozygotes and heterozygotes for the recessive model; 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 atherothrombotic cerebral infarction. The P-levels for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. In the stepwise forward selection procedure, each genotype was examined according to a dominant or recessive model on the basis of statistical significance in the multivariable logistic regression analysis. For all statistical analysis, a P-value of <0.05 was considered significant. Statistical significance was examined by two-sided tests, and statistical analysis was performed with JMP version 6.0 software (SAS Institute, Cary, NC, USA).

Results

The characteristics of the 1284 study subjects are shown in Table II. The frequency of male subjects, the prevalence of hypertension, hypercholesterolemia, and diabetes mellitus, systolic and diastolic blood pressure, and the percentage of glycosylated hemoglobin were greater, whereas BMI and the serum concentrations of triglycerides and HDL-cholesterol were lower, in subjects with atherothrombotic cerebral infarction than in controls.

Comparisons of genotype distributions with the Chi-square test revealed that the -786T→C polymorphism (rs2070744) of *NOS3*, the 2445G→A (Ala54Thr) polymorphism (rs1799883) of *FABP2*, the 190T→C (Trp64Arg) polymorphism (rs4994) of *ADRB3*, the 162A→C polymorphism (rs4769055) of *ALOX5AP*, the -413T→A polymorphism (rs2071746) of *HMOX1*, the A→G (Thr94Ala) polymorphism (rs2241883) of

Table IV. Hardy-Weinberg P-values in subjects with atherothrombotic cerebral infarction (ACI) and controls.

Gene	Polymorphism	ACI	Controls
NOS3	-786T→C	0.0014[a]	0.2905
FABP2	2445G→A (Ala54Thr)	0.2763	0.0892
ADRB3	190T→C (Trp64Arg)	0.0075[a]	0.2552
ALOX5AP	162A→C	0.6169	0.9410
HMOX1	-413T→A	0.2310	0.0037[a]
FABP1	A→G (Thr94Ala)	0.0137[a]	0.9121
THBS2	3949T→G (3'-UTR)	0.8994	0.4232
LTA4H	A→G (rs2660845)	0.0075[a]	0.4121
LGALS2	3279C→T (intron 1)	0.2390	0.6673
LIPC	-250G→A	0.0018[a]	0.9735
ADIPOQ	-11377C→G	0.4675	0.8969
LTA4H	A→G (rs2540482)	0.0098[a]	0.5345
ADIPOR2	795G→A	0.0451[a]	0.0002[a]
IPF1	-108/3G→4G	0.0221[a]	0.5845
LIPC	-514C→T	0.0054[a]	0.8470
ROS1	G→A (Asp2213Asn)	0.1064	0.1149
ROS1	G→C (Cys2229Ser)	0.0182[a]	0.8037

[a]P<0.05.

Table V. Multivariable logistic regression analysis of polymorphisms related to atherothrombotic cerebral infarction by the Chi-square test for individuals with metabolic syndrome.

Gene	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
NOS3	-786T→C	0.0851		0.0619		0.0253	0.65 (0.44-0.94)	0.0878	
FABP2	2445G→A (Ala54Thr)	0.0031	1.55 (1.16-2.07)	0.0048	1.75 (1.18-2.57)	0.0316	1.40 (1.03-1.91)	0.0007	2.08 (1.36-3.17)
ADRB3	190T→C (Trp64Arg)	0.2129		0.1318		0.0798		0.2096	
ALOX5AP	162A→C	0.0305	0.71 (0.52-0.97)	0.0028	0.59 (0.41-0.83)	0.2281		0.0015	0.51 (0.34-0.77)
FABP1	A→G (Thr94Ala)	0.0227	1.39 (1.05-1.84)	0.1291		0.0056	1.51 (1.13-2.02)	0.2983	
THBS2	3949T→G (3'-UTR)	0.1316		0.0206	6.49 (1.31-35.30)	0.2721		0.0187	6.68 (1.35-36.31)
LTA4H	A→G (rs2660845)	0.4756		0.2439		0.2234		0.9804	
LGALS2	3279C→T (intron 1)	0.2654		0.0209	0.54 (0.31-0.89)	0.6975		0.0197	0.53 (0.30-0.89)
LIPC	-250G→A	0.2322		0.1816		0.0667		0.9330	
ADIPOQ	-11377C→G	0.0867		0.0062	2.14 (1.23-3.68)	0.3341		0.0040	2.27 (1.29-3.95)
LTA4H	A→G (rs2540482)	0.7186		0.1928		0.3718		0.7866	
IPF1	-108/3G→4G	0.0065	0.65 (0.47-0.89)	0.6625		0.0062	0.62 (0.44-0.87)	0.0550	
LIPC	-514C→T	0.1194		0.4117		0.0459	0.71 (0.50-0.99)	0.6532	
ROS1	G→A (Asp2213Asn)	0.3800		0.0243	2.78 (1.13-6.83)	0.7660		0.0233	2.82 (1.14-6.94)
ROS1	G→C (Cys2229Ser)	0.0142	0.28 (0.10-0.78)	0.2284		0.0285	0.31 (0.11-0.89)	0.0129	0.28 (0.10-0.77)

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

Table VI. Effects of genotypes and other characteristics on atherothrombotic cerebral infarction among individuals with metabolic syndrome determined by a stepwise forward selection procedure (P<0.05).

Variable	P	R^2
Diabetes mellitus	<0.0001	0.0732
Hypertension	<0.0001	0.0419
BMI	<0.0001	0.0140
Hypercholesterolemia	0.0004	0.0107
Sex	0.0012	0.0089
FABP2 (*GA* + *AA* versus *GG*)	0.0037	0.0072
IPF1 (*3G4G* + *4G4G* versus *3G3G*)	0.0051	0.0067
FABP1 (*AG* + *GG* versus *AA*)	0.0063	0.0063
ROS1 (rs529038) (*AA* versus *GG* + *GA*)	0.0080	0.0060
ADIPOQ (*GG* versus *CC* + *CG*)	0.0082	0.0059
ALOX5AP (*CC* versus *AA* + *AC*)	0.0149	0.0050
NOS3 (*CC* versus *TT* + *TC*)	0.0237	0.0044
LGALS2 (*TT* versus *CC* + *CT*)	0.0405	0.0036

R^2, contribution rate.

FABP1, the 3949T→G polymorphism (rs8089) of *THBS2*, the A→G polymorphism (rs2660845) of *LTA4H*, the 3279C→T polymorphism (rs7291467) of *LGALS2*, the -250G→A polymorphism (rs2070895) of *LIPC*, the -11377C→G polymorphism (rs266729) of *ADIPOQ*, the A→G polymorphism (rs2540482) of *LTA4H*, the 795G→A polymorphism (rs16928751) of *ADIPOR2*, the -108/3G→4G polymorphism of *IPF1* (S82168), the -514C→T polymorphism (rs1800588) of *LIPC*, the G→A (Asp2213Asn) polymorphism (rs529038) of *ROS1*, and the G→C (Cys2229Ser) polymorphism (rs619203) of *ROS1* were related (P<0.05) to atherothrombotic cerebral infarction (Table III). The genotype distributions of these 17 polymorphisms in subjects with atherothrombotic cerebral infarction and in controls are also shown in Table III. In control subjects, the genotype distributions of these polymorphisms with the exception of those of *HMOX1* and *ADIPOR2* were in Hardy-Weinberg equilibrium (Table IV); the polymorphisms of *HMOX1* and *ADIPOR2* were therefore excluded from subsequent analysis.

Multivariable logistic regression analysis with adjustment for age, sex, BMI, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia revealed that the -786T→C polymorphism of *NOS3* (additive 1 model), the 2445G→A (Ala54Thr) polymorphism of *FABP2* (dominant, recessive, and additive 1 and 2 models), the 162A→C polymorphism of *ALOX5AP* (dominant, recessive, and additive 2 models), the A→G (Thr94Ala) polymorphism of *FABP1* (dominant and additive 1 models), the 3949T→G polymorphism of *THBS2* (recessive and additive 2 models), the 3279C→T polymorphism of *LGALS2* (recessive and additive 2 models), the -11377C→G polymorphism of *ADIPOQ* (recessive and additive 2 models), the -108/3G→4G polymorphism of *IPF1* (dominant and additive 1 models), the -514C→T polymorphism of *LIPC* (additive 1 model), the G→A (Asp2213Asn) polymorphism of *ROS1* (recessive and additive 2 models), and the G→C (Cys2229Ser) polymorphism of *ROS1* (dominant and additive 1 and 2 models) were associated (P<0.05) with the prevalence of atherothrombotic cerebral infarction (Table V). The variant *A* allele of *FABP2*, *G* allele of *FABP1*, *G* allele of *THBS2*, *G* allele of *ADIPOQ*, and *A* allele of the G→A (Asp2213Asn) polymorphism of *ROS1* were risk factors for atherothrombotic cerebral

infarction, whereas the variant *C* allele of *NOS3*, *C* allele of *ALOX5AP*, *T* allele of *LGALS2*, 4*G* allele of *IPF1*, *T* allele of the -514C→T polymorphism of *LIPC*, and *C* allele of the G→C (Cys2229Ser) polymorphism of *ROS1* were protective against this condition.

Finally, we performed a stepwise forward selection procedure to examine the effects of genotypes for the 11 polymorphisms associated with atherothrombotic cerebral infarction by multivariable logistic regression analysis as well as of age, sex, BMI, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia on atherothrombotic cerebral infarction (Table VI). Diabetes mellitus, hypertension, BMI, hypercholesterolemia, sex, *FABP2* genotype (dominant model), *IPF1* genotype (dominant model), *FABP1* genotype (dominant model), *ROS1* genotype (rs529038, recessive model), *ADIPOQ* genotype (recessive model), *ALOX5AP* genotype (recessive model), *NOS3* genotype (recessive model), and *LGALS2* genotype (recessive model), in descending order of statistical significance, were independent ($P<0.05$) determinants of atherothrombotic cerebral infarction.

Discussion

We examined the possible relations of 296 polymorphisms in 202 candidate genes to the prevalence of atherothrombotic cerebral infarction in 1284 Japanese individuals with metabolic syndrome. Our association study with three steps of analysis (Chi-square test, multivariable logistic regression analysis, and stepwise forward selection procedure) revealed that the 2445G→A (Ala54Thr) polymorphism of *FABP2*, the -108/3G→4G polymorphism of *IPF1*, the A→G (Thr94Ala) polymorphism of *FABP1*, the G→A (Asp2213Asn) polymorphism of *ROS1*, the -11377C→G polymorphism of *ADIPOQ*, the 162A→C polymorphism of *ALOX5AP*, the -786T→C polymorphism of *NOS3*, and the 3279C→T polymorphism of *LGALS2* were associated with the prevalence of atherothrombotic cerebral infarction. Among these polymorphisms, the 2445G→A (Ala54Thr) polymorphism of *FABP2* was most significantly associated with this condition.

Fatty acid-binding protein 2 (FABP2) is an intracellular protein that is expressed only in the columnar absorptive epithelial cells of the small intestine. It contains a single ligand site that has a high affinity for saturated and unsaturated fatty acids, and it contributes to the absorption and intracellular transport of long-chain fatty acids (17). The product of the *A* allele of the 2445G→A (Ala54Thr) polymorphism of *FABP2* possesses a greater affinity for long-chain fatty acids *in vitro* than does that of the *G* allele (18). In addition, individuals with the *A* allele of this polymorphism were found to be more insulin resistant than were those with the *G* allele (18,19). The *A* allele was also shown to be associated with higher plasma levels of low density lipoprotein-cholesterol (20) or with dyslipidemia (high plasma concentration of triglycerides and low concentration of HDL-cholesterol) (21). In addition, the *A* allele of the 2445G→A (Ala54Thr) polymorphism was previously associated with a parental history of stroke in the Swedish population (22). Moreover, it was associated with a 2- to 3.5-fold increase in cardiovascular risk in dyslipidemic men with diabetes compared with their dyslipidemic nondiabetic counterparts; for nonfatal myocardial infarction, stroke, or death from coronary heart disease, the corresponding hazard ratio was 3.0, whereas for stroke alone it was 3.5 (23). Our results show that the 2445G→A (Ala54Thr) polymorphism of *FABP2* was significantly associated with atherothrombotic cerebral infarction in individuals with metabolic syndrome, with the *A* (Thr) allele representing a risk factor for this condition. The effects of this polymorphism on both insulin resistance and lipid metabolism may account for its association with atherothrombotic cerebral infarction.

Among the seven polymorphisms of *IPF1*, *FABP1*, *ROS1* (rs529038), *ADIPOQ*, *ALOX5AP*, *NOS3*, and *LGALS2* also associated with atherothrombotic cerebral infarction in individuals with metabolic syndrome, the 162A→C polymorphism of *ALOX5AP* and the -786T→C polymorphism of *NOS3* have previously been associated with ischemic stroke (24,25). The -108/3G→4G polymorphism of *IPF1*, the G→A (Asp2213Asn) polymorphism of *ROS1*, the -11377C→G polymorphism of *ADIPOQ*, and the 3279C→T polymorphism of *LGALS2* were found not to be associated with ischemic stroke, but with myocardial infarction (26-30). The remaining A→G (Thr94Ala) polymorphism of *FABP1* has not been reported to be associated with ischemic stroke or myocardial infarction.

Given the multiple comparisons of genotypes with atherothrombotic cerebral infarction in the present study, it is not possible to exclude completely potential statistical errors such as false positives. It is also possible that one or more of the polymorphisms associated with this type of stroke in the present study are in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are actually responsible for the development of this condition. In addition, the functional relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study.

In conclusion, our present results suggest that *FABP2*, *IPF1*, *FABP1*, *ROS1*, *ADIPOQ*, *ALOX5AP*, *NOS3*, and *LGALS2* are susceptibility loci for atherothrombotic cerebral infarction among Japanese individuals with metabolic syndrome. Genotypes for these polymorphisms, especially for the 2445G→A (Ala54Thr) polymorphism of *FABP2*, may prove informative for assessment of genetic risk for atherothrombotic cerebral infarction among individuals with metabolic syndrome. Validation of our findings will require their replication with independent subject panels.

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