Assessment of genetic factors for type 2 diabetes mellitus

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Abstract. The purpose of the present study was to identify gene polymorphisms for reliable assessment of genetic factors for type 2 diabetes mellitus. The study population comprised 4853 unrelated Japanese individuals (2688 men, 2165 women), including 1489 subjects with type 2 diabetes mellitus (969 men, 520 women) and 3364 controls (1719 men, 1645 women). The genotypes for 148 polymorphisms of 124 candidate genes were determined with a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. Sixteen polymorphisms were related ($p<0.05$) to the prevalence of type 2 diabetes mellitus as determined by the chi-square test. Multivariable logistic regression analysis with adjustment for age, sex, and the prevalence of smoking revealed that, among these polymorphisms, the -603A→G polymorphism of the gene for coagulation factor III (*F3*) was significantly ($p<0.001$) associated with the prevalence of type 2 diabetes mellitus, with the -603G allele representing a risk factor for this condition. A stepwise forward selection procedure demonstrated that *F3* genotype (GG versus AA + AG) significantly ($p<0.001$) and independently affected the prevalence of type 2 diabetes mellitus. Genotype for *F3* may prove reliable for assessment of genetic factors for type 2 diabetes mellitus. Determination of the genotype for this gene may contribute to personalized prevention of this condition.

Introduction

Type 2 diabetes mellitus is a multifactorial disease with a substantial genetic component that is thought to be polygenic in nature. A combination of genes thus likely influences the underlying level of glucose intolerance in a population and thereby contributes to the overall susceptibility to type 2 diabetes mellitus. Although genetic linkage analyses (1-5) and association studies (6-10) have implicated several loci and candidate genes in predisposition to type 2 diabetes mellitus, the genes that contribute to genetic susceptibility to this condition remain to be identified definitively. In addition, given the ethnic differences in lifestyle and environmental factors as well as in genetic background, it is important to examine polymorphisms related to type 2 diabetes mellitus in each ethnic group.

We have now performed a large-scale association study for 148 candidate gene polymorphisms and type 2 diabetes mellitus in 4853 Japanese individuals. The purpose of the present study was to identify gene polymorphisms that confer susceptibility to type 2 diabetes mellitus and thereby to contribute to the personalized prevention of this condition.

Materials and methods

Study population. The study population comprised 4853 unrelated Japanese individuals (2688 men, 2165 women) who either visited outpatient clinics of or were admitted to one of the participating hospitals (Gifu Prefectural Gifu, Tajimi, and Gero Hotspring Hospitals; Hirosaki University Hospital; Reimeikyo Rehabilitation Hospital; and Yokohama General Hospital) between October 2002 and March 2005. The 1489 subjects (969 men, 520 women) with type 2 diabetes mellitus had a fasting plasma glucose concentration of ≥6.93 mmol/l (126 mg/dl) or a blood hemoglobin A_{1c} (HbA_{1c}) of ≥6.5% (or both) or were taking antidiabetes medication. Type 2 diabetes mellitus was defined according to the criteria accepted by the World Health Organization and described previously (11,12). Individuals with type 1 diabetes mellitus, with maturity-onset diabetes of the young, with other metabolic or endocrinologic diseases, or with severe liver or renal dysfunction were excluded from the study. Individuals taking drugs that cause secondary diabetes mellitus were also excluded.

The control subjects comprised a total of 3364 individuals (1719 men, 1645 women) who visited the outpatient clinics

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Table I. Characteristics of the 4853 study subjects.

Characteristic	Type 2 diabetes mellitus	Controls
No. of subjects	1489	3364
Age (years)	63.7±11.5	63.8±11.8
Sex (male/female, %)	65.1/34.9[a]	51.1/48.9
BMI (kg/m^2)	23.7±3.4[a]	23.3±2.8
Current or former smoker (%)	20.8[a]	16.2
Hypertension (%)	77.2[a]	49.6
Hypercholesterolemia (%)	51.6[a]	32.5
Fasting plasma glucose (mmol/l)	10.2±4.4[a]	5.2±0.7
HbA_{1c} (%)	7.8±2.2[a]	5.3±0.4

Data for age and body mass index (BMI) are means ± SD. Smoker: smoking of ≥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. [a]p<0.001 versus controls.

Table II. Polymorphisms related (p<0.05) to type 2 diabetes mellitus as determined by the chi-square test.

Gene symbol	Polymorphism	p-value
F3	-603A→G	0.0005
PON1	532A→G (Arg160Gly)	0.0026
ACE	-240A→T	0.0070
CD14	-260C→T	0.0074
ABCA1	2583A→G (Ile823Met)	0.0079
AP2M1	62G→T	0.0162
MMP12	-82A→G	0.0231
THBS2	3949T→G	0.0235
PPP1R3A	2711G→T (Tyr905Asp)	0.0312
F7	11,496G→A (Arg353Gln)	0.0314
PKD1-like	G→A (Gly243Asp)	0.0326
PECAM1	2201G→A (Gly670Arg)	0.0352
UTS2	347G→A (Ser89Asn)	0.0401
CX3CR1	926C→T (Thr280Met)	0.0410
AKAP10	2073A→G (Ile646Val)	0.0420
IPF1	-180/3G→4G	0.0457

of participating hospitals for an annual health checkup. They had a fasting plasma glucose concentration of <6.05 mmol/l (110 mg/dl) and a blood HbA_{1c} of <5.6%, and they had no history of diabetes mellitus or of taking antidiabetes medication. 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, and written informed consent was obtained from each participant.

Selection of polymorphisms. With the use of public databases, we selected 124 candidate genes that have been characterized and were suggested to be potentially associated with type 2 diabetes mellitus on the basis of a comprehensive overview of the function of pancreatic ß cells, peripheral insulin sensitivity, hepatic glucose production, lipid and adipose tissue metabolism, and other metabolic factors as well as of regulation of blood pressure and endocrine function, vascular biology, monocyte-macrophage biology, lymphocyte and other leukocyte biology, coagulation and fibrinolysis systems, and platelet function. We further selected for analysis 148 polymorphisms of these genes, most of which are located in the promoter region, exons, or splice donor or acceptor sites of introns and might therefore be expected to affect the function or expression of the encoded protein (Supplementary Table I).

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 148 polymorphisms were determined (G&G Science, Fukushima, Japan) by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Primers, probes, and other conditions for genotyping are shown in Supplementary Table II. The detailed genotyping methodology was described previously (13).

Statistical analysis. Clinical data were compared between subjects with type 2 diabetes mellitus 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 departures from Hardy-Weinberg equilibrium. The genotype distribution of each autosomal polymorphism was compared between subjects with type 2 diabetes mellitus and controls by the chi-square test (3x2); for polymorphisms on the X chromosome, allele frequencies were compared by the chi-square test (2x2). Polymorphisms related to type 2 diabetes mellitus (p<0.05) were examined by multivariable logistic regression analysis with adjustment for covariates, with type 2 diabetes mellitus as a dependent variable and independent variables including age, sex (0 = woman, 1 = man), smoking status (0 = nonsmoker, 1 = smoker), and genotype of each polymorphism. Each genotype was assessed according to dominant, recessive, and two additive (additive 1 and 2) genetic models, and the p-value, odds ratio, and 95% confidence interval were calculated. 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 type 2 diabetes

Table III. Multivariable logistic regression analysis of polymorphisms related to type 2 diabetes mellitus.

Gene symbol	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)
F3	-603A→G	0.8103		**0.0001**	1.75 (1.32-2.33)	0.1574		**0.0003**	1.70 (1.27-2.26)
PON1	532A→G (Arg160Gly)	0.0039	1.28 (1.08-1.52)	0.2203		0.0020	1.31 (1.10-1.55)	0.2451	
ACE	-240A→T	0.0042	1.20 (1.06-1.37)	0.0575		0.0169	1.18 (1.03-1.35)	0.0077	1.30 (1.07-1.57)
CD14	-260C→T	0.0040	1.25 (1.07-1.46)	0.2578		0.0077	1.24 (1.06-1.46)	0.0105	1.26 (1.06-1.50)
ABCA1	2583A→G (Ile823Met)	0.0344	1.22 (1.02-1.46)	0.0040	1.20 (1.06-1.36)	0.2104		0.0050	1.32 (1.09-1.61)
AP2M1	62G→T	0.0813		0.0044	1.35 (1.10-1.65)	0.3746		0.0029	1.38 (1.12-1.71)
MMP12	-82A→G	0.6391		0.5840		0.4346		0.5843	
THBS2	3949T→G	0.0137	1.22 (1.04-1.42)	0.0736		0.0351	1.19 (1.01-1.40)	0.0578	
PPP1R3A	2711G→T (Tyr905Asp)	0.0341	1.28 (1.02-1.62)	0.6659		0.0183	1.34 (1.05-1.70)	0.0766	
F7	11,496G→A (Arg353Gln)	0.0226	0.80 (0.65-0.97)	0.6628		0.0167	0.78 (0.64-0.95)	0.7049	
PKD1-like	G→A (Gly243Asp)	0.0472	0.48 (0.22-0.95)			0.0472	0.48 (0.22-0.95)		
PECAM1	2201G→A (Gly670Arg)	0.0442	1.17 (1.00-1.36)	0.3118		0.0134	1.23 (1.04-1.44)	0.4209	
UTS2	347G→A (Ser89Asn)	0.1204		0.1228		0.0393	1.15 (1.01-1.31)	0.2283	
CX3CR1	926C→T (Thr280Met)	0.0113	1.31 (1.06-1.62)	0.9138		0.0103	1.32 (1.07-1.63)	0.9365	
AKAP10	2073A→G (Ile646Val)	0.0414	0.87 (0.77-0.99)	0.0838		0.1056		0.0537	
IPF1	-180/3G→4G	0.0316	0.85 (0.74-0.99)	0.1380		0.0806		0.0254	0.82 (0.69-0.98)

Multivariable logistic regression analysis was performed with adjustment for age, sex, and the prevalence of smoking. OR, odds ratio; CI, confidence interval. p-values of <0.001 are shown in bold.

mellitus. The levels for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. Given the multiple comparisons of genotypes with type 2 diabetes mellitus, we adopted a strict criterion ($p<0.001$) for statistical significance of association in order to avoid type I error. 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 version 5.1 software (SAS Institute, Cary, NC, USA).

Results

The characteristics of the 4853 study subjects are shown in Table I. The frequency of male subjects, body mass index, and the prevalence of smoking, hypertension, and hypercholesterolemia were greater for subjects with type 2 diabetes mellitus than for controls. Comparison of genotype distributions with the chi-square test revealed that 16 polymorphisms were related ($p<0.05$) to the prevalence of type 2 diabetes mellitus (Table II). These polymorphisms were further analyzed for their possible association with type 2 diabetes mellitus.

Multivariable logistic regression analysis with adjustment for age, sex, and smoking status revealed that the -603A→G polymorphism of the coagulation factor III gene (*F3*, recessive and additive 2 models) was significantly ($p<0.001$) associated with the prevalence of type 2 diabetes mellitus, with the -603G allele being a risk factor for this condition (Table III). The genotype distributions for all 16 polymorphisms related to type 2 diabetes are shown in Table IV; those in control subjects were in Hardy-Weinberg equilibrium.

Finally, we performed a stepwise forward selection procedure to examine the effects of genotypes for these polymorphisms, age, sex, and smoking status on type 2 diabetes mellitus (Table V). Sex and *F3* genotype (recessive model), in descending order of statistical significance ($p<0.001$), independently influenced the prevalence of type 2 diabetes mellitus.

Discussion

We have examined the possible relationships of 148 polymorphisms in 124 candidate genes to type 2 diabetes mellitus. Our large-scale association study with 4853 individuals revealed that the -603A→G polymorphism of *F3* was significantly associated with the prevalence of type 2 diabetes mellitus in the Japanese population. The chromosomal region containing *F3* (1p22-21) has not previously been linked to type 2 diabetes mellitus in the Japanese population (14-16), and *F3* itself has not been identified as a candidate gene for predisposition to this condition.

F3 (tissue factor or tissue thromboplastin) is a 47-kDa transmembrane glycoprotein, which, in response to binding of factor VIIa, activates coagulation factor X by converting it to factor Xa and thereby initiates the extrinsic coagulation cascade. Although F3 is normally not expressed in circulating leukocytes or endothelial cells, transcription of *F3* is induced in these cells by proinflammatory cytokines, growth factors, shear forces, or balloon injury of the vessel wall (17). The abundance of F3 mRNA is increased in several tissues, including adipose tissue, of obese mice compared with those

Table IV. Genotype distributions of polymorphisms related to type 2 diabetes mellitus.

Gene symbol	Polymorphism	Type 2 diabetes mellitus	Controls
F3	-603A→G		
	AA	64.7	64.8
	AG	29.3	31.6
	GG	6.0	3.5
PON1	532A→G (Arg160Gly)		
	AA	83.1	86.4
	AG	16.7	13.1
	GG	0.2	0.5
ACE	-240A→T		
	AA	36.8	41.4
	AT	48.3	45.7
	TT	14.9	12.9
CD14	-260C→T		
	CC	19.5	23.5
	CT	50.9	48.6
	TT	29.6	28.0
ABCA1	2583A→G (Ile823Met)		
	AA	12.6	14.9
	AG	44.6	46.7
	GG	42.8	38.4
AP2M1	62G→T		
	GG	47.8	50.5
	GT	41.2	41.0
	TT	11.0	8.5
MMP12	-82A→G		
	AA	96.1	95.9
	AG	3.7	4.1
	GG	0.2	0
THBS2	3949T→G		
	TT	79.7	82.5
	TG	18.7	16.6
	GG	1.6	0.9
PPP1R3A	2711G→T (Tyr905Asp)		
	GG	7.3	9.4
	GT	42.6	40.4
	TT	50.2	50.2
F7	11,496G→A (Arg353Gln)		
	GG	89.6	87.1
	GA	9.9	12.5
	AA	0.5	0.4
PKD1-like	G→A (Gly243Asp)		
	GG	99.4	98.8
	GA	0.6	1.3
	AA	0	0
PECAM1	2201G→A (Gly670Arg)		
	GG	19.5	22.0
	GA	52.9	49.1
	AA	27.5	28.9
UTS2	347G→A (Ser89Asn)		
	GG	59.4	61.9
	GA	36.5	33.2
	AA	4.0	5.0
CX3CR1	926C→T (Thr280Met)		
	CC	89.8	92.0
	CT	10.1	7.9
	TT	0.1	0.2
AKAP10	2073A→G (Ile646Val)		
	AA	66.2	62.8
	AG	30.4	32.7
	GG	3.4	4.5
IPF1	-180/3G→4G		
	3G3G	25.3	22.2
	3G4G	49.3	50.4
	4G4G	25.4	27.4

of lean mice (18,19), suggesting that hyperinsulinemia associated with insulin-resistant states, such as obesity and type 2 diabetes mellitus, may induce *F3* expression locally in multiple tissues.

The expression of F3 was shown to be higher in monocytes from individuals with diabetes mellitus than in those from nondiabetic controls (20). Although similar numbers of cell-derived microparticles were found in both individuals with well-controlled, uncomplicated type 2 diabetes mellitus and nondiabetic controls, a higher proportion of microparticles derived from T helper cells, granulocytes, and platelets exposed F3 in the former group (21). The plasma concentration of F3 was also higher in individuals with type 2 diabetes mellitus than in nondiabetic controls; furthermore, it was higher in diabetic individuals with cardiovascular disease than in those without this condition, suggesting that F3 levels are related to vascular complications (22,23). Indeed, the -603A→G polymorphism of *F3* has been associated with myocardial infarction, with the -603G allele being a risk factor for this condition (24). Moberg *et al* (25) showed that an instant blood-mediated inflammatory reaction (IBMIR) occurs frequently during transplantation of pancreatic islets. Given that F3 is produced and secreted by the endocrine cells of islets of Langerhans and that the IBMIR is inhibited by antibodies to F3 and site-inactivated factor VIIa *in vitro*, these researchers concluded that the IBMIR is triggered by

Table V. Effects of genotypes and other characteristics on type 2 diabetes mellitus as determined by a stepwise forward selection procedure.

Variable	p-value	R^2
Sex	<0.0001	0.0138
F3 (GG versus AA + AG)	0.0002	0.0024
ACE (TT + AT versus AA)	0.0031	0.0015
PON1 (GG + AG versus AA)	0.0040	0.0014
CD14 (TT + CT versus CC)	0.0040	0.0014
AP2MI (TT versus GG + GT)	0.0043	0.0013
ABCA1 (GG versus AA + AG)	0.0061	0.0012
CX3CR1 (TT + CT versus CC)	0.0099	0.0012
IPF1 (4G4G + 3G4G versus 3G3G)	0.0194	0.0009
THBS2 (GG + TG versus TT)	0.0231	0.0009
F7 (AA + GA versus GG)	0.0243	0.0008
PPP1R3A (TT + GT versus GG)	0.0262	0.0008
PECAM1 (AA + GA versus GG)	0.0346	0.0008
PKD1-like (AA + GA versus GG)	0.0379	0.0007

F3. These observations suggest that inhibition of F3 activity may be beneficial during clinical islet transplantation.

Induction of F3 expression occurs at a transcriptional level through the action of cell type-specific promoters. Transcriptional induction of *F3* is mediated by AP-1 and NF-κB sites in endothelial and monocytic cells and by Egr-1 and Sp1 sites in epithelial and smooth muscle cells and monocytes (17). Various promoter polymorphisms of *F3* have been described: four polymorphisms (-1812C→T, -1322C→T, -1208D/I, and -603A→G) are in linkage disequilibrium and two other polymorphisms (-1442G→C and -21C→T) are rare (26). Individuals homozygous for the D allele of the deletion/insertion (D/I) polymorphism at nucleotide position -1208 were found to have a lower circulating level of F3 than those homozygous for the I allele (26). The -603A→G polymorphism was associated with the abundance of F3 mRNA in monocytes, with individuals with the G allele manifesting a larger amount of F3 mRNA than those with the AA genotype (27). We have now shown that the -603A→G polymorphism of *F3* is significantly associated with the prevalence of type 2 diabetes mellitus, with the -603G allele being a risk factor for this condition. This is the first demonstration of an association of a polymorphism of *F3* with type 2 diabetes mellitus, although the underlying molecular mechanism remains to be elucidated. Our finding is consistent with previous observations that the levels of F3 in plasma and in various types of cells are increased in diabetic subjects, that increased levels of F3 are deleterious in terms of vascular complications in diabetic individuals and for islet transplantation, and that the G allele of the-603A→G polymorphism is related to increased expression of F3 (20-23,25).

Given the multiple comparisons of genotypes with type 2 diabetes mellitus in the present study, we adopted a strict criterion ($p<0.001$) for statistical significance of association. It is not possible, however, to exclude completely potential statistical errors such as false positives. It is also possible that the -603A→G polymorphism of *F3* is in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for the development of type 2 diabetes mellitus. The functional relevance of the -603A→G polymorphism of *F3* to the pathophysiology of type 2 diabetes mellitus was also not examined in the present study. Despite these limitations, our present results suggest that *F3* is a susceptibility locus for type 2 diabetes mellitus in the Japanese population. Determination of genotype for this polymorphism may prove informative for assessment of the genetic risk for type 2 diabetes mellitus and may contribute to the personalized prevention of this condition.

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Supplementary Table I. The 148 polymorphisms examined in the study.

Locus	Gene	Symbol	Polymorphism	dbSNP[a]
1p36.3	5,10-Methylenetetrahydrofolate reductase	*MTHFR*	677C→T (Ala222Val)	rs1801133
1p36	Urotensin II	*UTS2*	347G→A (Ser89Asn)	rs2890565
1p34.2	Polycystic kidney disease 1-like	*PKD1-like*	G→A (Gly243Asp)	rs1635712
1p34.1-p32	Proprotein convertase, subtilisin/kexin-type, 9	*PCSK9*	23968A→G (Glu670Gly)	rs505151
1p22-p21	Coagulation factor III	*F3*	-603A→G	rs1361600
1p22.1	Glutamate-cysteine ligase, modifier subunit	*GCLM*	-588C→T	(U72210)
1q23-q25	Selectin E	*SELE*	561A→C (Ser128Arg)	rs5361
1q23-q25	Selectin P	*SELP*	G→T (Val640Leu)	rs6133
1q25	Tumor necrosis factor ligand superfamily, member 4	*TNFSF4*	A→G	rs3850641
1q31-q32	Interleukin 10	*IL10*	-819T→C	rs1800871
1q31-q32	Interleukin 10	*IL10*	-592A→C	rs1800872
1q42-q43	Angiotensinogen	*AGT*	-6G→A	rs5051

Supplementary Table I. Continued.

Locus	Gene	Symbol	Polymorphism	dbSNP[a]
2q14	Interleukin 1-ß	*IL1B*	-511C→T	rs16944
2q36	Insulin receptor substrate 1	*IRS1*	3931G→A (Gly972Arg)	rs1801278
2q37.3	Calpain 10	*CAPN10*	4852G→A	rs3792267
3pter-p21	Chemokine, CX3C motif, receptor 1	*CX3CR1*	926C→T (Thr280Met)	rs3732378
3p25	Peroxisome proliferator-activated receptor-γ	*PPARG*	-681C→G	rs10865710
3p25	Peroxisome proliferator-activated receptor-γ		34C→G (Pro12Ala)	rs1801282
3p22	Transforming growth factor-ß receptor, type II	*TGFBR2*	1167C→T (Asn389Asn)	rs2228048
3p22-p21.3	Phospholipase C, δ-1	*PLCD1*	864G→A (Arg257His)	rs933135
3p21.3	Glutathione peroxidase	*GPX1*	C→T (Pro198Leu)	rs1050450
3p21	Chemokine, CC motif, receptor 2	*CCR2*	190G→A (Val64Ile)	rs1799864
3p21	Chemokine, CC motif, receptor 5	*CCR5*	59029G→A	rs1799987
3q21-q25	Angiotensin receptor 1	*AGTR1*	1166A→C	rs5186
3q21-q25	Angiotensin receptor 1	*AGTR1*	G→A (Ala163Thr)	rs12721226
3q24-q25	Purinergic receptor P2Y, G protein-coupled, 12	*P2RY12*	744T→C	(NC_000003)
3q26.1-q26.2	Butyrylcholinesterase	*BCHE*	1615G→A (Ala539Thr)	rs1803274
3q26.3-q27	Thrombopoietin	*THPO*	5713A→G	rs6141
3q27	Adipocyte, C1Q, and collagen domain containing	*ACDC*	-11377C→G	rs266729
3q28	Adaptor-related protein complex 2, MU-1 subunit	*AP2M1*	62G→T	rs1501299
4p15.1	Peroxisome proliferator-activated receptor-γ, coactivator 1	*PPARGC1*	1564G→A (Gly482Ser)	rs8192678
4q22-q24	Microsomal triglyceride transfer protein, 88-kD	*MTP*	-493G→T	rs1800591
4q26-q28	Annexin A5	*ANXA5*	-1C→T	rs11575945
4q28-q31	Fatty acid-binding protein 2	*FABP2*	2445G→A (Ala54Thr)	rs1799883
4q31	Uncoupling protein 1	*UCP1*	-112A→C	rs10011540
4q31.22	Endothelin receptor, type A	*EDNRA*	-231A→G	rs1801708
5q12	Phosphodiesterase 4D, cAMP-specific	*PDE4D*	TAAA→- (3'-UTR)	rs3839219
5q13	Thrombospondin IV	*THBS4*	1186G→C (Ala387Pro)	rs1866389
5q13	Phosphatidylinositol 3-kinase, regulatory, 1	*PIK3R1*	1020G→A (Met326Ile)	rs3730089
5q23-q31	Integrin, α-2	*ITGA2*	1648A→G (Lys505Glu)	rs10471371
5q31.1	Monocyte differentiation antigen CD14	*CD14*	-260C→T	rs2569190
5q32-q34	ß-2-adrenergic receptor	*ADRB2*	46A→G (Arg16Gly)	rs1042713
5q32-q34	ß-2-adrenergic receptor	*ADRB2*	79C→G (Gln27Glu)	rs1042714
5q33-qter	Factor XII	*F12*	46C→T	rs17876008
6p24-p23	Endothelin 1	*EDN1*	5665G→T (Lys198Asn)	rs5370
6p21.3	Lymphotoxin-α	*LTA*	804C→A (Thr26Asn)	rs2229093
6p21.3	Tumor necrosis factor	*TNF*	-863C→A	rs1800630
6p21.3	Tumor necrosis factor	*TNF*	-850C→T	rs1799724
6p21.3	Tumor necrosis factor	*TNF*	-238G→A	rs361525
6p21.3	Advanced glycosylation end product-specific receptor	*AGER*	268G→A (Gly82Ser)	rs2070600
6p21.2-p21.1	Peroxisome proliferator-activated receptor-δ	*PPARD*	294T_C	rs2016520
6p21.2-p12	Phospholipase A2, group VII	*PLA2G7*	994G→T (Val279Phe)	rs16874954
6p12	Glutamate-cysteine ligase, catalytic subunit	*GCLC*	-129C→T	rs17883901
6p12	Vascular endothelial growth factor	*VEGF*	936C→T	rs3025039
6q22-q23	Ectonucleotide pyrophosphatase/phosphodiesterase 1	*ENPP1*	97A→C (Lys121Gln)	rs1044498
6q25.1	Estrogen receptor 1	*ESR1*	-1989T→G	rs2071454
6q27	Thrombospondin II	*THBS2*	3949T→G	rs8089
7p21	Interleukin 6	*IL6*	-572G→C	rs1800796
7p15-p13	Glucokinase	*GCK*	-30G→A	(M90297)
7q11.2	Syntaxin 1A	*STX1A*	205T→C (Asp68Asp)	rs2293485

Supplementary Table I. Continued.

Locus	Gene	Symbol	Polymorphism	dbSNP[a]
7q11.2	CD36 antigen	*CD36*	30294G→C	rs1049673
7q11.23-q21.11	Protein phosphatase 1, regulatory subunit 3A	*PPP1R3A*	2647G→T (Ser883Arg)	(X78578)
7q11.23-q21.11	Protein phosphatase 1, regulatory subunit 3A	*PPP1R3A*	2711G→T (Tyr905Asp)	rs1799999
7q21.3	Paraoxonase 1	*PON1*	-162G→A	rs705381
7q21.3	Paraoxonase 1	*PON1*	532A→G (Arg160Gly)	rs13306698
7q21.3	Paraoxonase 1	*PON1*	584G→A (Gln192Arg)	rs662
7q21.3	Paraoxonase 2	*PON2*	475C→G (Ala148Gly)	rs11545941
7q21.3-q22	Plasminogen activator inhibitor 1	*PAI1*	-668/4G→5G	rs1799768
7q21.3-q22	Plasminogen activator inhibitor 1	*PAI1*	A→G (Tyr243Cys)	rs13306846
7q32	Paired box gene 4	*PAX4*	567C→T (Arg121Trp)	(AF043978)
7q36	Nitric oxide synthase 3	*NOS3*	-786T→C	rs2070744
8p22	Lipoprotein lipase	*LPL*	1595C→G (Ser447Stop)	rs328
8p21-p12	Epoxide hydrolase 2, cytosolic	*EPHX2*	G→A (Arg287Gln)	rs751141
8p12	Plasminogen activator, tissue	*PLAT*	-7351C→T	rs2020918
8p12-p11.2	ß-3-adrenergic receptor	*ADRB3*	190T→C (Trp64Arg)	rs4994
8p12-p11.2	RecQ protein-like 2	*RECQL2*	47765T→C (Cys1367Arg)	rs1346044
9q22-q31	ATP-binding cassette, subfamily A, member 1	*ABCA1*	1051G→A (Arg219Lys)	rs2230806
9q22-q31	ATP-binding cassette, subfamily A, member 1	*ABCA1*	2583A→G (Ile823Met)	rs4149313
9q34.1	Endoglin	*ENG*	1691C→G (Asp366His)	rs1800956
9q34.2-q34.3	Prostaglandin D2 synthase, brain	*PTGDS*	4111A→C	rs6926
10q11.2	Arachidonate 5-lipoxygenase	*ALOX5*	G→A (Glu254Lys)	rs2228065
10q24-q26	ß-1-adrenergic receptor	*ADRB1*	1165G→C (Gly389Arg)	rs1801253
11p15.5	Insulin	*INS*	-23T→A	rs689
11p15.1	Potassium channel, inwardly rectifying, subfamily J, member 11	*KCNJ11*	276A→G (Glu23Lys)	rs5219
11p15.1	ATP-binding cassette, subfamily C, member 8	*ABCC8*	3857G→A (Arg1273Arg)	rs4148643
11q13	Uncoupling protein 2	*UCP2*	-866G→A	rs659366
11q13	Uncoupling protein 3	*UCP3*	-55C→T	rs1800849
11q22.2-q22.3	Matrix metalloproteinase 12	*MMP12*	-82A→G	rs2276109
11q22-q23	Matrix metalloproteinase 1	*MMP1*	-1607/1G→2G	rs1799750
11q23	Apolipoprotein A-I	*APOA1*	-75G→A	rs670
11q23	Apolipoprotein A-I	*APOA1*	84T→C	rs5070
11q23	Apolipoprotein A-V	*APOA5*	-1131T→C	rs662799
11q23	Apolipoprotein C-III	*APOC3*	-482C→T	rs2854117
11q23	Apolipoprotein C-III	*APOC3*	1100C→T	rs4520
11q23	Matrix metalloproteinase 3	*MMP3*	-1171/5A→6A	rs3025058
11q23	Matrix metalloproteinase 3	*MMP3*	A→G (Lys45Glu)	rs679620
11q23.3-q25	Heat-shock 70-kD protein 8	*HSPA8*	-110A→C	rs1008438
12p13	Guanine nucleotide-binding protein, ß-3	*GNB3*	825C→T (splice variant)	rs5443
12p13-p12	Low density lipoprotein, oxidized, receptor 1	*OLR1*	501G→C (Lys167Asn)	rs11053646
13q12.1	Insulin promoter factor 1	*IPF1*	-108/3G→4G	(S82168)
13q14.11	Carboxypeptidase B2, plasma	*CPB2*	529G→A (Ala147Thr)	rs3742264
13q14.11	Carboxypeptidase B2, plasma	*CPB2*	T→C (Ile347Thr)	rs1926447
13q34	Factor VII	*F7*	11496G→A (Arg353Gln)	rs6046
14q11.2	Cathepsin G	*CTSG*	2108A→G (Asn125Ser)	(J04990)
14q32.1	α-1-antichymotrypsin	*AACT*	50G→A (Ala15Thr)	rs4934
14q32.1-q32.2	Bradykinin receptor B2	*BDKRB2*	C→T (Arg14Cys)	rs1046248
15q21-q23	Lipase, hepatic	*LIPC*	-250G→A	rs2070895

Supplementary Table I. Continued.

Locus	Gene	Symbol	Polymorphism	dbSNP[a]
16p13	Major histocompatibility complex, class II, transactivator	*MHC2TA*	-168A→G	rs3087456
16q13	Matrix metalloproteinase 2	*MMP2*	-1306C→T	rs243865
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	-629C→A	rs1800775
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	1061A→G (Ile405Val)	rs5882
16q24	Cytochrome b(-245), α subunit	*CYBA*	242C→T (His72Tyr)	rs4673
17pter-p12	Glycoprotein Ib, platelet, α polypeptide	*GP1BA*	-5T→C	rs2243093
17pter-p12	Glycoprotein Ib, platelet, α polypeptide	*GP1BA*	1018C→T (Thr145Met)	rs6065
17p13	Chemokine, CXC motif, ligand 16	*CXCL16*	C→T (Ala181Val)	rs2277680
17p11.2	Sterol regulatory element-binding transcription factor 1	*SREBF1*	-36G→-	(AX977070)
17p11.1	A-kinase anchoring protein 10	*AKAP10*	2073A→G (Ile646Val)	rs203462
17q11.2-q12	Chemokine, CC motif, ligand 5	*CCL5*	-28C→G	rs2280788
17q11.2-q12	Chemokine, CC motif, ligand 5	*CCL5*	-403G→A	rs2107538
17q21.1-q21.2	Chemokine, CC motif, ligand 11	*CCL11*	G→A (Ala23Thr)	rs3744508
17q23	Angiotensin I- converting enzyme	*ACE*	-240A→T	rs4291
17q23	Platelet-endothelial cell adhesion molecule 1	*PECAM1*	1454C→G (Leu125Val)	rs668
17q23	Platelet-endothelial cell adhesion molecule 1	*PECAM1*	2201G→A (Gly670Arg)	rs1131012
17q23-qter	Apolipoprotein H	*APOH*	341G→A (Ser88Asn)	rs1801692
18q21.1	Lipase, endothelial	*LIPG*	584C→T (Thr111Ile)	rs2000813
19p13.3	Resistin	*RETN*	-420C→G (C-180G)	rs1862513
19p13.3	Resistin	*RETN*	-180C→G	rs1862513
19p13.3	Resistin	*RETN*	+62G→A	rs3745368
19p13.3-p13.2	Intercellular adhesion molecule 1	*ICAM1*	1462G→A (Glu469Lys)	rs5498
19p13.2	Insulin receptor	*INSR*	7067365C→A	rs2860172
19p13.2	Low density lipoprotein receptor	*LDLR*	1184G→A (Ala370Thr)	rs11669576
19q13.1	Transforming growth factor, ß-1	*TGFB1*	-509C→T	rs1800469
19q13.2	Apolipoprotein E	*APOE*	-219G→T	rs405509
19q13.2	Apolipoprotein E	*APOE*	3932T→C (Cys112Arg)	rs429358
19q13.2	Apolipoprotein E	*APOE*	4070C→T (Arg158Cys)	rs7412
19q13.3	Glycogen synthase 1	*GYS1*	260A→G (Met416Val)	rs5447
19q13.4	Glycoprotein VI, platelet	*GP6*	13254T→C (Ser219Pro)	rs1613662
20p11.2	Thrombomodulin	*THBD*	2136C→T (Ala455Val)	rs1042579
20q11.2-q13.1	Matrix metalloproteinase 9	*MMP9*	855G→A (Arg279Gln)	rs2664538
20q13.11-q13.13	Prostaglandin I2 synthase	*PTGIS*	1117C→A	rs6095558
20q13.31	Phosphoenolpyruvate carboxykinase 1, soluble	*PCK1*	-232C→G	rs2071023
21q22.3	Integrin, ß-2	*ITGB2*	1323C→T	rs235326
22q11.2	Catechol-O-methyltransferase	*COMT*	G→A (Val158Met)	rs4680
22q12	Heme oxygenase 1	*HMOX1*	-413T→A	rs2071746
22q12	Heme oxygenase 1	*HMOX1*	99G→C (Asp7His)	rs2071747
22q12-q13	Lectin, garactoside-binding, soluble, 2	*LGALS2*	3279C→T (intron 1)	rs7291467
Xq22-q23	Angiotensin II receptor, type 2	*AGTR2*	1675G→A	rs1403543
Xq22-q23	Angiotensin II receptor, type 2	*AGTR2*	3123C→A	rs11091046

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

Supplementary Table II. Primers, probes, and other PCR conditions for genotyping.

Gene Symbol	Polymorphism	Sense primer	Antisense primer	Probe 1	Probe 2	Annealing (°C)	Cycles (times)
F3	-603A→G	TCTCCTgTgCgACCCgCTAAg	AgCCACggTggCTTCTTCTAC	gTgggCAggCCAAgTATTCT	AggTCAAgAATACCTggCCT	60	50
PON1	532A→G (Arg160Gly)	ATCCAgATgCCAAgTCCACAgT	gTggATTAACTATCCgCTACAgC	TCTAAAAACCATCAgACATAAAC	CAgAAgTTTATgTCCgATggTT	60	50
ACE	-240A→T	gCTCgggTgTTCCggCAAACT	ggCTCCCgCAgAggAAgCTg	ggTCCCCATCTTCAAAAgAgAg	CTCCTCTCTTTAgAAgATggg	60	50
CD14	-260C→T	CCTAgATgCCCTgCAgAATCC	TggAAATATTgCAATgAAggATgTT	CTTCCTgTTACggCCCCCCTC	TTCCTgTTACggTCCCCCTC	60	50
ABCA1	2583A→G (Ile823Met)	AggAAAgTgATgAgAAgAgCCAC	CAgAggCAgCAgCACTAggTTA	TTACTTTCTgATATTCTCTTC	CACTTACTTTCTgACATTCTCT	60	50
AP2M1	62G→T	TCATCACAgACCTCCTACACTg	TTTCTCCCTgTgTCTAggCCTT	CTTAgTTAATAATgAATgCCTTCATA	ACTATATgAAgTCATTCATTATTAA	60	50
MMP12	-82A→G	CTAATTgATCCATTgTCgTCTgAAT	CTCTTTATATAgCCCTTAgTCCg	ggATgATATCAACTATgAgTCA	ggATgATATCAACTgTgAgTCA	60	50
THBS2	3949T→G	AACCCAAgTgCCTTCAgAggAT	CTCCACATAAAgTCTCATATATCAC	gATgTTCATCTCTgAgTTCCA	gATgTTCATCTCTgCgTTCCA	60	50
PPP1R3A	2711G→T (Tyr905Asp)	AACAgACTCggATgCCATTgTg	TTgACACTgAAATTTCAgTATgATg	ATTAgTgTCTgAgTTAAAAgCA	CTCTATTAgTgTATgAgTTAAA	60	50
F7	11496G→A (Arg353Gln)	CggCTACTCggATggCAgCA	CCAAAgTggCCCACggTTgC	TACCACgTgCCCCggTAgTg	gCCACCCACTACCAgggCA	60	50
PKD1-like	G→A (Gly243Asp)	TCCCCTAACCACAgACCTgAC	TCTgATATTTCAggTTgCACTgAT	CATTCTTTggCCCACCAgACA	CATTCTTTggCCCATCAgACA	60	50
PECAM1	2201G→A (Gly670Arg)	gAATTTCCCTTgTCACTCACCCTA	gATAATTACCTTTATTATCATTTATTgg	ATTgCATggTTTCCgACATCg	AATgACgATgTCAgAAACCATgC	60	50
UTS2	347G→A (Ser89Asn)	TACAAgAgAAACAACAgATCTgATg	CTAACTCATAAATAgAgTCACTTAC	TTAAAAATgTTggTACTTgAgTCT	TTAAAAATgTTggTATTTgAgTCT	60	50
CX3CR1	926C→T (Thr280Met)	ACTTCTTTCCCAgTTgTgACATg	TCCCCAgCAAATgCATAgATgAg	AgTgTgACTgAgACggTTgCA	ggCTAAATgCAACCATCTCAgT	60	50
AKAP10	2073A→G (Ile646Val)	ggCCCAggAAgAgCTAgCTTg	gTAgATTTCTCTAACggTTgATCAT	gATAgTCAgTgACATTATgCAg	CCTgCTgCATAACgTCACTg	60	50
IPF1	-180/3G→4G	TggCTgTgggTTCCCTCTgAg	gATTTggCACTgTgTggCgTTC	CgAgCAggggTggCgCC	ggCgCCACCCTgCTCgCT	60	50