Genetic factors for obesity

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Abstract. The purpose of the present study was to identify gene polymorphisms for the reliable assessment of genetic factors for obesity. The study population comprised 3906 unrelated Japanese individuals (2286 men, 1620 women), including 1196 subjects (677 men, 519 women) with obesity (body mass index of ≥25 kg/m^2) and 2710 controls (1609 men, 1101 women). The genotypes for 147 polymorphisms of 124 candidate genes were determined with a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. Multivariable logistic regression analysis with adjustment for age, sex, and the prevalence of smoking revealed that the -30G→A polymorphism of *GCK*, the -240A→T polymorphism of *ACE*, and the -482C→T polymorphism of *APOC3* were significantly (P<0.01) associated with the prevalence of obesity, and the -1989T→G polymorphism of *ESR1* was almost significantly associated. A stepwise forward selection procedure demonstrated that *ACE*, *GCK*, and *ESR1* genotypes significantly (P<0.01) and independently affected the prevalence of obesity. Combined genotype analysis for these three polymorphisms yielded a lowest odds ratio of 0.45 for the combined genotypes of *AT* or *TT* for *ACE*, *GG* for *GCK*, and *GG* for *ESR1* in comparison with the combined genotypes of *AA* for *ACE*, *GG* for *GCK*, and *TT* or *TG* for *ESR1*. Genotypes for *ACE*, *GCK*, and *ESR1* may prove reliable for the assessment of genetic factors for obesity. Determination of the combined genotypes for these genes may contribute to the personalized prevention of this condition.

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Introduction

The prevalence of obesity, a multifactorial disease caused by an interaction of genetic factors with lifestyle and environmental factors (1), is rapidly increasing worldwide. A sedentary lifestyle, high-fat and high-energy diet, and genetic predisposition to obesity all contribute to the epidemic. Although genetic linkage analyses (2-5) and candidate gene approaches (6-9) have implicated several loci and candidate genes in predisposition to obesity, 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 gene polymorphisms related to obesity in each ethnic group.

We have now performed a large-scale association study for 147 candidate gene polymorphisms and obesity in 3906 Japanese individuals. The purpose of the present study was to identify gene polymorphisms for the reliable assessment of the genetic factors for obesity, and thereby to contribute to the personalized prevention of this condition.

Materials and methods

Study population. The study population comprised 3906 unrelated Japanese individuals (2286 men, 1620 women) who either visited outpatient clinics at or were admitted to one of the five participating hospitals (Gifu Prefectural Gifu Hospital, Gifu Prefectural Tajimi Hospital, Gifu Prefectural Gero Hotspring Hospital, Hirosaki University Hospital, and Reimeikyo Rehabilitation Hospital) between October 2002 and March 2005. Obesity was defined as a body mass index (BMI) of ≥25 kg/m^2 on the basis of the BMI criteria for Japanese and Asian populations (10). A total of 1196 individuals (677 men, 519 women) among the study population were thus classified as obese. The controls comprised a total of 2710 individuals (1609 men, 1101 women) who visited the outpatient clinics of the participating hospitals for an annual health checkup and who had a BMI of <25 kg/m^2. 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

Table I. Characteristics of the 3906 study subjects.

Characteristic	Obesity	Controls
No. of subjects	1196	2710
Age (years)	63.0±10.2[a]	65.7±11.1
Sex (male/female) (%)	56.6/43.4	59.4/40.6
Body mass index (kg/m^2)	27.4±2.2[a]	21.8±2.1
Current or former smoker (%)	18.8	18.2
Hypertension (%)	68.2[b]	63.0
Systolic blood pressure (mmHg)	149±28[a]	145±28
Diastolic blood pressure (mmHg)	82±15[a]	79±16
Hypercholesterolemia (%)	50.7[a]	43.0
Total cholesterol (mmol/l)	5.56±1.09[a]	5.33±1.04
HDL-cholesterol (mmol/l)	1.31±0.47[a]	1.39±0.36
Triglycerides (mmol/l)	1.85±1.25[a]	1.53±1.05
Diabetes mellitus (%)	38.6[c]	34.7
Fasting plasma glucose (mmol/l)	7.32±3.96[b]	6.93±3.58
Glycosylated hemoglobin (%)	6.4±1.9[d]	6.2±1.8

Nonprevalence data are means ± SD. HDL, high density lipoprotein. Smoker, ≥10 cigarettes daily. Hypertension, systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg (or both), or taking antihypertensive medication. Diabetes mellitus, fasting plasma glucose ≥6.93 mmol/l or glycosylated hemoglobin ≥6.5% (or both), or taking antidiabetes medication. Hypercholesterolemia, serum total cholesterol ≥5.72 mmol/l or taking lipid-lowering medication. [a]P<0.001, [b]P<0.005, [c]P <0.05, [d]P<0.01 versus controls.

School of Medicine, Gifu International Institute of Biotechnology, and the 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 suggested to be associated with obesity on the basis of a comprehensive overview of: lipid and adipose tissue metabolism, insulin and glucose metabolism, other metabolic factors as well as the regulation of blood pressure and endocrine function, vascular biology, monocyte-macrophage biology, lymphocyte and leukocyte biology, coagulation and fibrinolysis systems and platelet function. We further selected 147 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 result in changes in the function or expression of the encoded protein (Supplementary Table I).

Table II. Polymorphisms related (P<0.05) to obesity as evaluated by the Chi-square test.

Gene symbol	Polymorphism	P
GCK	-30G→A	0.0079
ACE	-240A→T	0.0102
APOC3	-482C→T	0.0173
GCLC	-129C→T	0.0298
ESR1	-1989T→G	0.0302
STX1A	205T→C (Asp68Asp)	0.0336
IRS1	3931G→A (Gly972Arg)	0.0344
APOA1	-75G→A	0.0345
F12	46C→T	0.0462

Genotyping of polymorphisms. Venous blood (7 ml) was collected in tubes containing 50 mmol/l EDTA (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 147 polymorphisms were determined (G&G Science, Fukushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX). Primers, probes, and other conditions for genotyping are shown in Supplementary Table II. Detailed genotyping methodology was described previously (11).

Statistical analysis. Clinical data were compared between the subjects with obesity and the controls by the unpaired Student's t-test. Qualitative data were compared by the Chi-square test.

Table III. Multivariable logistic regression analysis of polymorphisms related to obesity.

Gene symbol	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
GCK	-30G→A	**0.0049**	0.81 (0.70-0.94)	0.0477	0.64 (0.40-0.98)	0.0171	0.83 (0.71-0.97)	0.0268	0.60 (0.38-0.93)
ACE	-240A→T	**0.0026**	0.81 (0.70-0.93)	0.5651		**0.0029**	0.80 (0.69-0.93)	0.1038	
APOC3	-482C→T	**0.0077**	1.24 (1.06-1.45)	0.8496		**0.0038**	1.28 (1.08-1.51)	0.1491	
GCLC	-129C→T	0.5313		0.0119	1.89 (1.14-3.12)	0.2103		0.0157	1.85 (1.12-3.05)
ESR1	-1989T→G	0.3119		0.0106	0.76 (0.61-0.93)	0.8691		0.0135	0.75 (0.60-0.94)
STX1A	205T→C (Asp68Asp)	0.1103		0.0411	1.21 (1.01-1.46)	0.3279		0.0237	1.27 (1.03-1.55)
IRS1	3931G→A (Gly972Arg)	0.0119	1.46 (1.08-1.95)	0.9370		0.0109	1.47 (1.09-1.97)	0.9507	
APOA1	-75G→A	0.0369	0.85 (0.73-0.99)	0.0644		0.1020		0.0442	0.65 (0.42-0.98)
F12	46C→T	0.5399		0.0396	1.16 (1.01-1.33)	0.1738		0.8367	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, and the prevalence of smoking. P values of <0.01 are shown in bold.

Table IV. Genotype distribution of polymorphisms related to obesity.

Gene symbol	Polymorphism	Obesity	Controls
GCK	-30G→A		
	GG	70.5	66.0
	GA	27.3	30.6
	AA	2.2	3.4
ACE	-240A→T		
	AA	43.0	37.9
	AT	43.7	48.2
	TT	13.3	13.9
APOC3	-482C→T		
	CC	24.5	28.5
	CT	52.7	48.4
	TT	22.8	23.1
GCLC	-129C→T		
	CC	76.6	75.8
	CT	21.0	22.9
	TT	2.4	1.3
ESR1	-1989T→G		
	TT	43.9	42.2
	TG	45.2	43.9
	GG	10.9	13.9
STX1A	205T→C (Asp68Asp)		
	TT	36.1	39.0
	TC	46.7	46.8
	CC	17.2	14.2
IRS1	3931G→A (Gly972Arg)		
	GG	93.4	95.4
	GA	6.5	4.5
	AA	0.1	0.1
APOA1	-75G→A		
	GG	70.9	67.6
	GA	26.6	28.7
	AA	2.5	3.8
F12	46C→T		
	CC	12.4	11.6
	CT	41.9	46.2
	TT	45.7	42.3

Allele frequencies were estimated by the gene counting method, and the Chi-square test was used to identify departures from Hardy-Weinberg equilibrium. In the initial screening, the genotype distribution of each autosomal polymorphism was compared between the subjects with obesity and the 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 obesity ($P<0.05$) were further examined by multivariable logistic regression analysis with adjustment for covariates, with obesity 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 additive (1 and 2) genetic models, and the P value, odds ratio, and 95% confidence interval were calculated. The additive genetic model 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. For combined genotype analysis, multivariable logistic regression analysis was performed with obesity as a dependent variable and independent variables including age, sex, smoking status, and combined genotypes. Each genotype was assessed according to a dominant or recessive model based on statistical significance, and each combined genotype was compared to that which conferred the highest genetic risk for obesity. We also performed a stepwise forward selection procedure to examine the effects of genotypes as well as of other covariates on obesity. The levels for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. Given the multiple comparisons of genotypes with obesity, we adopted a level of $P<0.01$ for statistical significance of 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 version 5.1 software (SAS Institute, Cary, NC).

Table V. Effects of genotypes and other characteristics on obesity as determined by a stepwise forward selection procedure ($P<0.05$).

Variable	P	R^2
Age	<0.0001	0.0104
ACE (TT + AT versus AA)	0.0027	0.0019
GCK (AA + GA versus GG)	0.0052	0.0016
ESR1 (GG versus TT + TG)	0.0098	0.0014
APOC3 (TT + CT versus CC)	0.0110	0.0014
IRS1 (AA + GA versus GG)	0.0114	0.0013
GCLC (TT versus CC + CT)	0.0142	0.0012
F12 (TT versus CC + CT)	0.0376	0.0009
STX1A (CC versus TT + TC)	0.0446	0.0008

Results

The characteristics of the 3906 study subjects are shown in Table I. Subjects with obesity were younger and exhibited a higher prevalence of hypertension, hypercholesterolemia, and diabetes mellitus compared with the controls. Systolic and diastolic blood pressure, the serum concentrations of total cholesterol and triglycerides, the fasting plasma concentration of glucose, and the serum level of glycosylated hemoglobin were higher, and the serum concentration of high density lipoprotein-cholesterol was lower, in the subjects with obesity than in the controls. Comparison of genotype distributions with the Chi-square test revealed that nine polymorphisms were related ($P<0.05$) to the prevalence of obesity (Table II). These polymorphisms were further analyzed for their possible association with obesity.

Multivariable logistic regression analysis with adjustment for age, sex, and the prevalence of smoking revealed that the -30G→A polymorphism of the glucokinase gene (*GCK*, dominant model), the -240A→T polymorphism of the

Table VI. Assessment of genetic risk for obesity with three combined genotypes.

ACE (0=AA, 1=AT=TT)	*GCK* (0=GG, 1=GA=AA)	*ESR1* (0=TT=TG, 1=GG)	No. of subjects (obesity/controls)	OR (95% CI)	P
1	0	1	45/173	0.45 (0.31-0.64)	0.00001
1	1	1	23/76	0.51 (0.31-0.82)	0.00750
0	1	0	123/328	0.65 (0.50-0.83)	0.00070
1	1	0	189/479	0.68 (0.55-0.84)	0.00050
1	0	0	425/955	0.77 (0.65-0.92)	0.00480
0	1	1	18/39	0.80	0.44420
0	0	1	44/89	0.87	0.46600
0	0	0	329/571	1.00	

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

angiotensin-converting enzyme gene (*ACE*, dominant and additive 1 models), and the -482C→T polymorphism of the apolipoprotein C-III gene (*APOC3*, dominant and additive 1 models) were significantly (P<0.01) associated with the prevalence of obesity (Table III). In addition, the -1989T→G polymorphism of the estrogen receptor α gene (*ESR1*, recessive model) was almost significantly associated with obesity. The -482*T* allele of *APOC3* represented a risk factor for obesity, whereas the -30*A* allele of *GCK*, the -240*T* allele of *ACE*, and the -1989*G* allele of *ESR1* were protective against this condition. The genotype distributions of the nine identified polymorphisms were in Hardy-Weinberg equilibrium in both the controls and the subjects with obesity (Table IV).

We next performed a stepwise forward selection procedure to examine the effects of genotypes for these polymorphisms, age, sex, and smoking status on obesity (Table V). Age, *ACE* genotype (dominant model), *GCK* genotype (dominant model), and *ERS1* genotype (recessive model), in descending order of statistical significance (P<0.01), each independently affected the prevalence of obesity.

Finally, we calculated the odds ratio, 95% confidence interval, and P value for combined genotypes in assessment of the genetic risk for obesity. Combined genotype analysis of three polymorphisms (-240A→T in *ACE*, -30G→A in *GCK*, and -1989T→G in *ESR1*) revealed that a lowest odds ratio of 0.45 was obtained for the combined genotype of *AT* or *TT* for *ACE*, *GG* for *GCK*, and *GG* for *ESR1* in comparison with the combined genotype of *AA* for *ACE*, *GG* for *GCK*, and *TT* or *TG* for *ESR1* (Table VI).

Discussion

We have examined the relations of 147 polymorphisms in 124 candidate genes to obesity. Our large-scale association study with 3906 subjects revealed that the -240A→T polymorphism of *ACE*, the -30G→A polymorphism of *GCK*, and the -1989T→G polymorphism of *ESR1* were significantly associated with the prevalence of obesity in a Japanese population. Combined genotype analysis of these three polymorphisms yielded a lowest odds ratio of 0.45 for the predisposition to obesity.

The renin-angiotensin system of adipose tissue plays an important role in adipocyte growth and differentiation through the action of angiotensin II (12,13). In addition, epidemiologic studies have demonstrated associations between the plasma concentration of angiotensinogen (14,15), plasma renin activity (16), or plasma ACE activity (17) with BMI. In adult white men, homozygosity for the *D* allele of an insertion/deletion (I/D) polymorphism in intron 16 of *ACE* was associated with a greater prevalence of age-related abdominal adiposity and with a greater tendency to become overweight during 20 years of follow-up, consistent with a role for the local renin-angiotensin system in adipose tissue metabolism and, more generally, with a genetic influence on the control of fat deposition (18). The I/D polymorphism of *ACE* was also associated with obesity and abdominal fat deposition in subjects with coronary heart disease; subjects with this condition and the *D* allele showed a higher prevalence of obesity and abdominal fat deposition as well as higher values for weight and waist circumference (19). A haplotype in the promoter region of *ACE* was transmitted preferentially from parents to offspring who became obese among black populations in both the United States and Nigeria, suggesting that *ACE* polymorphisms may influence the development of weight gain (20). We have now shown that the -240A→T polymorphism of *ACE* was significantly associated with the prevalence of obesity, while the *T* allele protected against this condition. Our results are thus consistent with the previous observations that *ACE* polymorphisms are related to obesity.

Glucokinase is expressed in pancreatic ß cells and hepatocytes, its expression being controlled by two tissue-specific promoters (21). Pancreatic glucokinase serves as the sensor for glucose in the regulation of insulin secretion (22). Mutations of *GCK* account for 10-50% of cases of maturity-onset diabetes of the young (22). A-30G→A polymorphism located in the ß cell-specific promoter of *GCK* was shown to be associated with reduced ß cell function and impaired glucose tolerance in Japanese (23,24). We have now shown that this polymorphism is associated with obesity, with the *A* allele being protective against this condition. The mechanisms responsible for the association of the *A* variant with reduced

ß cell function and impaired glucose tolerance (23,24) as well as with a reduced risk of obesity (our study) remain to be determined. It is possible that the -30G→A polymorphism of *GCK* is in linkage disequilibrium with other polymorphisms of nearby genes which are actually responsible for obesity.

A lack of ESR1 resulted in white adipocyte hyperplasia and hypertrophy, insulin resistance, and glucose intolerance in both sexes of mice (25). Estrogen-ESR1 signaling thus plays an important role in white adipose tissue of males and females; obesity in ESR1-deficient male mice resulted from reduced energy expenditure rather than increased energy intake. Two single nucleotide polymorphisms have been identified in the first intron of *ESR1*: a T→C polymorphism that is recognized by the restriction endonuclease *Pvu* II and an A→G polymorphism that is recognized by *Xba* I [*A* and *G* alleles correspond to the presence (*x* allele) and absence (*X* allele) of the restriction site, respectively]. The *GG* (*XX*) genotype was found to be significantly more frequent among subjects with type 2 diabetes mellitus and android-type obesity than in healthy individuals (26). The *GG* (*XX*) genotype of the *Xba* I polymorphism was also shown to contribute to the development of android-type fat distribution in middle-aged and premenopausal Japanese women (27). We have now shown that the -1989T→G polymorphism of *ESR1* was significantly associated with the prevalence of obesity, with the *G* allele protecting against this condition. The -1989T→G polymorphism is in linkage disequilibrium with the *Xba* I polymorphism in Japanese individuals, with the *G* allele of the former polymorphism being associated with the *A* (*x*) allele of the latter (28). The previous observations that the *GG* (*XX*) genotype is related to android-type obesity are thus consistent with our present results.

Given the multiple comparisons of genotypes with obesity in the present study, we adopted a strict criterion ($P<0.01$) for the statistical significance of association. It is not possible, however, to completely exclude potential statistical errors such as false positives. It is also possible that one or more of the polymorphisms associated with obesity 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 relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study. Despite these limitations, our present results suggest that *ACE*, *GCK*, and *ESR1* are susceptibility loci for obesity in the Japanese population. Determination of combined genotypes for these polymorphisms may prove informative for assessment of the genetic factors for obesity and may contribute to the personalized prevention of this condition.

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Supplementary Table I. The 147 polymorphisms examined in the present study.

Locus	Gene	Symbol	Polymorphism	dbSNP[a]
1p36.3	5,10-Methylenetetrahydrofolate reductase	*MTHFR*	677C→T (Ala222Val)	rs1801133
1p36.2	Natriuretic peptide precursor A	*NPPA*	664G→A (Val7Met)	rs5063
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)
1q21-q23	C-reactive protein, pentraxin-related	*CRP*	1444C→T	rs1130864
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
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	*PPARG*	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	Fibrinogen, B ß polypeptide	*FGB*	-455G→A	rs1800790
4q28	Fibrinogen, B ß polypeptide	*FGB*	8059G→A (Arg448Lys)	rs4220
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
5q32-q34	ß-2-adrenergic receptor	*ADRB2*	46A→G (Arg16Gly)	rs1042713
5q32-q34	ß-2-adrenergic receptor	*ADRB2*	79C→G (Gln27Glu)	rs1042714

Supplementary Table I. Continued.

Locus	Gene	Symbol	Polymorphism	dbSNP[a]
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
6p21	Solute carrier family 26 (sulfate transporter), member 8	*SLC26A8*	A→G (Ile639Val)	rs2295852
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	rs1799884
7q11.2	Syntaxin 1A	*STX1A*	205T→C (Asp68Asp)	rs2293485
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
1q23	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)

Supplementary Table I. Continued.

Locus	Gene	Symbol	Polymorphism	dbSNP[a]
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
13q34	Protein Z	*PROZ*	79G→A	rs3024735
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
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)
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-qter	Apolipoprotein H	*APOH*	341G→A (Ser88Asn)	rs1801692
18q21.1	Lipase, endothelial	*LIPG*	584C→T (Thr111Ile)	rs2000813
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 the event that 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)
GCK	-30G→A	ATggTCAgCCCTgCTgAggC	ggCATTTCCTgCTCCAgCCAg	AgggCTTACTgTgCTCCTgA	AgggCTTACTgTgTTCCTgA	60	50
ACE	-249A→T	gCTCgggTgTTCCggCAAACT	ggCTCCCgCAgAggAAgCTg	ggTCCCCATCTTCAAAAgAgAg	CTCCTCTCTTTAgAAgATggg	60	50
APOC3	-482C→T	AggggCTgTgAgAgCTCAgC	AggggCTTCTTCAgACTTgAgA	ggCACAgAAgACCAggCATCA	gCCACTgATgCCCggTCTTC	60	50
GCLC	-129C→T	gCATTTTgATATgTCgCgTTTgC	CggAggCgTggCCTgAAgC	CTCAACTgCgACCCAATCA	gggTgATTgggTCACAgTTgAg	60	50
ESR1	-1989T→G	CCgAATCCCTgCCATTCCACC	ggAAggAATgTgCTCgCATgT	ACATCCACACACTCTCTCTgC	TAggCAgAgAgCgTgTgTgg	60	50
STX1A	205T→C (Asp68Asp)	AAgCggAAgCACAgTgCCATC	gAggCTTgTggggCCTgAAAC	CACACTCACTCTCATCggg	CCAACCCCgACgAgAgTgA	60	50
IRS1	3931G→A (Gly972Arg)	AggAgAgCACTggggTCgAg	ggACAACTCATCTgCATggTCAT	TgCTAgCAgCCCCgggAgg	CTgCACCTCCCAgggCTgCTA	60	50
APOA1	-75G→A	ggACAgAgCTgATCCTTgAACT	gCAgggCCTATTTATgTCTgCA	TAAgCCCAgCCCCggCCCT	TAAgCCCAgCCCTggCCCT	60	50
F12	46C→T	ggCAgCTTgACCAATCTCTATT	TCCTggTTCCCACAgCACTCA	CAACggACggACgCCATgAg	AgCCCTCATggCATCCgTCC	60	50