PHARMACOGENETICS AND GENOMICS

A study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with type 2 diabetes mellitus

Background and Objective: **Troglitazone is a 2,4-thiazolidinedione antidiabetic agent with insulin-sensitizing activities. This agent had been used efficiently in a large number of patients but was withdrawn from the market in March 2000 because of its association with idiosyncratic hepatotoxicity. To address the susceptible genetic factors responsible for the hepatotoxicity associated with this agent, we performed a genetic polymorphic analysis by a target gene approach in troglitazone-treated Japanese patients with type 2 diabetes mellitus.**

Methods: **One hundred ten patients treated with troglitazone were recruited into this study. The case patients (n = 25) were recruited through medical professionals who had previously reported abnormal increases in the levels of ALT or AST among their patients. The control patients (n = 85) were recruited through physicians prescribing troglitazone. For statistical accuracy, efforts were made to maximize the size of the case group. Genotype analysis was performed in 68 polymorphic sites of 51 candidate genes related to drug metabolism, apoptosis, production and elimination of reactive oxygen species, and signal transduction pathways of peroxisome proliferator–activated receptor gamma 2 and insulin.**

Results: **The strong correlation with transaminase elevations was observed in the combined glutathione-S-transferase *GSTT1-GSTM1* null genotype (odds ratio, 3.692; 95% confidence interval, 1.354-10.066; *P* = .008).**

Conclusions: **The double null mutation of *GSTT1* and *GSTM1* might influence troglitazone-associated abnormal increases of liver enzyme levels. (Clin Pharmacol Ther 2003;73:435-55.)**

Ichiro Watanabe, PhD, Aiko Tomita, Miho Shimizu, Mie Sugawara, Hiroaki Yasumo, Ryuta Koishi, PhD, Tohru Takahashi, PhD, Kaoru Miyoshi, Kouichi Nakamura, PhD, Takashi Izumi, PhD, Yasuyuki Matsushita, Hidehiko Furukawa, PhD, Hideyuki Haruyama, PhD, and Teiichiro Koga, PhD *Tokyo, Japan*

Thiazolidinediones are a new class of oral antidiabetic agents that directly target insulin resistance. Among them, troglitazone (Noscal, Sankyo Co, Ltd, Tokyo, Japan) ([±]-5-[4-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-ylmethoxy)-benzyl]-2,4-thiazolidinedione) was the first thiazolidinedione developed to treat type 2 diabetes mellitus.[1-3] This agent has a novel mechanism of action, increasing insulin sensitivity, lowering blood glucose levels through increased glucose uptake by skeletal muscles and decreased hepatic

From the Biomedical Research Laboratories, Pharmacokinetics and Drug Delivery Research Laboratories, and Regulatory Affairs Department, Sankyo Co, Ltd.

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Reprint requests: Teiichiro Koga, PhD, Biomedical Research Laboratories, Sankyo Co, Ltd, 2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan.

E-mail: *kogasa@shina.sankyo.co.jp*

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glucose production, and decreasing glycosylated hemoglobin 1A, serum triglyceride, and circulating insulin levels.[4-9] In animal models of diabetes including Zucker fatty rats and KK, *ob/ob,* and *db/db* mice, this agent lowered the levels of plasma glucose, insulin, ketone bodies, triglycerides, and plasma lactate.[1,3,10,11] Although the mechanisms by which cellular metabolic changes take place in patients with diabetes are not fully understood, this class of agent is thought to activate peroxisome proliferator–activated receptor gamma (PPARγ), which leads to the increased expression of different proteins regulating glucose and lipid metabolism such as CD36, leptin, and adiponectin at the transcription levels. These proteins amplify the postreceptor signaling of insulin actions in the liver and peripheral tissues, leading to improvement of glycemic control.[5,8,9]

Troglitazone was introduced into the market in early 1997 and has been used effectively in a large number of patients with type 2 diabetes.[4-8] This agent had acceptable safety profiles in clinical trials,[2,6] but after its launch, troglitazone-associated sporadic hepatic dysfunction and hepatic failure were reported.[12-18] However, the liver dysfunction associated with this agent was unpredictable from experimental animal studies because standard animal models did not reveal clinical or pathologic symptoms of liver changes, even in long-term studies.[19-21] Despite troglitazone's effectiveness and great benefit to patients with diabetes, as a consequence of safety concerns over its use, this agent was withdrawn from the US and Japanese market in March 2000.

Great efforts have been made to elucidate the mechanisms for troglitazone-associated hepatotoxicity. Toyoda et al[22] have reported that troglitazone causes apoptotic cell death in primary rat hepatocytes cultured in serum-free medium. In contrast, Ogata et al[23] have demonstrated that this cytotoxicity of troglitazone in cultured rat hepatocytes is induced only under nonphysiologic conditions such as serum-free medium and that the cytotoxicity is not relevant to liver toxicity in vivo. Haskins et al[24] have demonstrated that thiazolidinediones, such as troglitazone, pioglitazone, and rosiglitazone, have direct effects on mitochondria in isolated rat and human hepatocytes and cause alterations of mitochondrial activity. These thiazolidinediones also caused hepatic enzyme leakage, decreased reductive metabolism, and cytoplasmic adenosine triphosphate depletion. All of these hepatic alterations in vitro are characteristics of thiazolidinediones, with only quantitative differences in subcellular organelle change. Funk et al[25,26] have examined the cholestatic potential of troglitazone and its major metabolite, troglitazone sulfate, and demonstrated that troglitazone sulfate was responsible for the interaction with the hepatobiliary export of bile acids in rats, which might lead to a troglitazone-induced intrahepatic cholestasis, thereby potentially contributing to the formation of hepatotoxicity. In contrast, Kostrubsky et al[27] have shown that in cultured human and pig hepatocytes the accumulation of troglitazone and the reduction of troglitazone sulfation correlated with increases in toxicity. Despite all of these efforts, the molecular basis for underlying troglitazone-associated hepatotoxicity has not been fully clarified.

The mechanisms of drug-induced liver injury can be classified into intrinsic and idiosyncratic.[28,29] In some cases of troglitazone-associated hepatitis, liver biopsies were performed and demonstrated that the hepatocellular nature of the injury was consistent with an idiosyncratic drug reaction.[10] Kuramoto et al[13] have also reported that the hepatotoxicity of troglitazone might be associated with idiosyncratic abnormalities in drug metabolism.

We performed an association study of troglitazone-treated Japanese patients with type 2 diabetes to survey the susceptible genetic factors responsible for the hepatotoxicity. We genotyped the 68 polymorphic loci of 51 different genes in 110 Japanese patients with type 2 diabetes, consisting of 25 case patients and 85 control patients. The case patients were recruited through medical professionals who had previously reported abnormal increases in the levels of ALT or AST among their patients. The control patients were recruited through physicians prescribing troglitazone. The data obtained indicate that the presence of the combined null genotype for glutathione-S-transferase (GST) theta 1 (*GSTT1*) and GST mu 1 (*GSTM1*) might influence troglitazone-associated abnormal increases in liver enzyme levels.

MATERIAL AND METHODS

Subjects. One hundred ten troglitazone-treated Japanese patients (47 men and 63 women) with type 2 diabetes were recruited in this study. All patients provided informed consent at the commencement of the study. Subsequently, the study was reviewed by the Ethical Committee of Sankyo Co, Ltd, which had not been established at the time of the commencement of the study, and was found to comply with the intent of the Declaration of Helsinki. The case patients were recruited through medical professionals who had previously reported abnormal increases in the levels of ALT and AST among their patients. The control patients were recruited through physicians prescribing troglitazone. To improve statistical comparisons, we

made efforts to recruit as many patients for the case group as possible. However, because of the rarity of troglitazone-associated abnormal increases in liver enzyme levels observed clinically, it was not feasible to have equal numbers of case patients and control patients. Twenty-five case patients had an abnormal increase in ALT levels (peak values ranged from 360 to 1905 IU/L, at least 9 times the upper limit of the normal range) or AST levels (peak values ranged from 184 to 1467 IU/L, at least 5 times the upper limit of the normal range) during the troglitazone treatment, whereas 85 control patients showed no remarkable increase in ALT levels (≤39 IU/L) or AST levels (≤35 IU/L) for more than 6 months of troglitazone treatment. ALT and AST values before troglitazone treatment were not available for 9 case patients and 11 control patients. Although 1 patient in the control group did not have the peak ALT and AST values during the treatment period, we included that patient in the control group according to the judgment of the physician in charge. For all other patients, liver enzyme levels before administration of troglitazone were within the normal ranges. For 1 patient in the case group, no information in terms of dose was available, whereas the remainder took 200 to 400 mg/d troglitazone.

Materials and apparatus. Oligonucleotides for primers were obtained from Amersham Pharmacia (Tokyo, Japan). *LA Taq* deoxyribonucleic acid (DNA) polymerases (catalog No. RR02AG) were purchased from Takara Shuzo (Tokyo, Japan). Genomic DNA was extracted from white blood cells by the NA1000 DNA-extracting apparatus (Kurabo Industries Ltd, Osaka, Japan). DNA concentration was determined photometrically with the use of Gene Spec I (Hitachi, Tokyo, Japan).

Genotyping of candidate genes. Fifty-one different genes related to drug metabolism, apoptosis, production and elimination of reactive oxygen species, and signal transduction pathways of PPARγ2 and insulin were selected as candidates for analysis. Genotypes at polymorphic sites for the following 20 genes were determined in the case (n = 25) and control (n = 85) patients: cytochrome P450 (CYP) (*CYP1A1*, *CYP2C9*, *CYP2C19*, *CYP2E1*, and *CYP3A4*); uridine diphosphate–glucuronosyltransferase 1A1 (*UGT1A1*), *GSTT1*, and *GSTM1*, glutathione peroxidase (GPX) 1 (*GPX3* and *GPX4*), catalase (*catalase*), nicotinamide adenine dinucleotide phosphate, reduced:quinone acceptor oxidoreductase (*DT-diaphorase*), tumor necrosis factor α (*TNFα*), *PPARγ2*, β_3-adrenergic receptor (*ADRB3*), uncoupling protein 1 (*UCP1*), superoxide dismutase 1 (*SOD1*), multidrug resistance–associated protein 2 (*MRP2*), and glucose transporter 1 (*GLUT1*). We typed an additional 31 genes, including those for thiopurine methyltransferase (*TPMT*), caspase 9 (*CASP9*), Fas antigen (*Fas antigen*), cytotoxic T-lymphocyte–associated molecule 4 (*CTLA4*), retinol dehydrogenase 5 (*RDH5*), tumor necrosis factor receptor (*TNFR1* and *TNFR2*), nitric oxide synthase (*NOS2A* and *NOS3*), monoamine oxidase B (*MAOB*), cytochrome c oxidase 2 (*MTCO2*), p22 phox (*p22 phox*), selenoprotein P (*SEP*), hepatocyte growth factor (*HGF*), leptin (*LEP*), leptin receptor (*LEPR*), albumin (*ALB*), apolipoprotein (*APOA1* and *APOC3*), lipoprotein lipase (*LPL*), CD36 (*CD36*), insulin receptor (*IR*), insulin-like growth factor (*IGF1* and *IGF2*), insulin-like growth factor receptor 2 (*IGFR2*), insulin receptor substrate (*IRS1*, *IRS2*, and *IRS4*), glucose transporter 2 (*GLUT2*), and glycogen synthase (*GYS1* and *GYS2*), in 103 patients, consisting of case patients (n = 23) and control patients (n = 80). Genotyping of these 31 genes was an additional step and was therefore performed in those 103 patients who consented to reanalysis for these genes.

Genotyping of DNA. Genotyping of DNA was performed by direct sequencing, with the exception of *GSTT1* and *GSTM1* null genotyping as mentioned later. Polymorphic regions were amplified by polymerase chain reaction (PCR) with specific primers. (The primer sequences are listed in Appendix Table I.) The PCR was conducted in a 0.02-mL reaction mixture containing 1 ng/μL genomic DNA, 500 nmol/L sense/antisense primers, 0.4 mmol/L of each deoxynucleotide triphosphate, and 0.05 U/μL *LA Taq* DNA polymerase in 1× *LA Taq* DNA polymerase buffer (Gene Amp 9700; Applied Biosystems, Foster City, Calif). The PCR was done by 35 or 40 cycles of 30 seconds at 94°C (degeneration), 30 seconds at 50°C to 65°C (annealing), and 30 seconds at 72°C (elongation).

Genotyping of GSTT1 and GSTM1. Null genotyping of *GSTT1* and *GSTM1* was determined by agarose electrophoresis. The *GSTT1* and *GSTM1* genes were amplified by the PCR in the same reaction mixture described here containing specific primer sets for *GSTT1* or *GSTM1*. (The primer sequences are listed in Appendix Table I.) The PCR was done by 35 cycles of 30 seconds at 94°C (degeneration), 30 seconds at 60°C (annealing), and 30 seconds at 72°C (elongation). The amplified DNA fragments were electrophoresed in 1.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet illumination. When the PCR products gave 0.46-kilobase (kb) or 0.23-kb bands, they were designated as wild type for *GSTT1* or *GSTM1* genes, respectively, and when no amplified band was seen, they were designated as null genotype. The genotyping for *CYP2C19* m1 was performed si-

Table I. Male/female ratio and age distribution among case and control groups

	First subset (n = 110)				*Second subset (n = 103)*			
	Group		*Probability*		*Group*		*Probability*	
Characteristic	*Control*	*Case*	χ^2 *Value*	*P value*	*Control*	*Case*	χ^2 *Value*	*P value*
Age (y)	85	25			80	23		
30-39	1	0			1	0		
40-49	11	3			10	3		
50-59	24	8	1.344	.930	24	8	1.569	.905
60-69	31	9			28	7		
70-79	15	5			14	5		
80-89	3	0			3	0		
Sex								
Male	40	7	2.868	.090	36	6	2.646	.104
Female	45	18			44	17		

multaneously in each PCR amplification as a positive control of PCR.

Statistical analysis. If it is assumed that the study would show a 15% incidence of genotype mutation among control patients and a 50% incidence among case patients, then a minimum number of 20 case patients and 50 control patients would be required to achieve a statistical power of 80% at the .05 significance level (2-sided). Chi-square analysis was performed to test the significant differences in the male-to-female ratio; the distribution of age, genotype, and allele frequency; and Hardy-Weinberg equilibrium between the case and control groups. A logistic regression model was used to estimate odds ratio (OR) and its 95% confidence interval (CI). Statistical analysis was performed with SAS (version 6.12; SAS Institute Japan Ltd, Tokyo, Japan). $P < .05$ was considered statistically significant.

RESULTS

Patient background data. Table I shows the background data of 110 patients recruited into the first subset of the genotyping study and 103 patients included in the second subset of the genotyping study. In both studies, no significant differences in the male-to-female ratio and the distribution of ages were observed between the case and control groups.

Polymorphic analysis. In this study we analyzed 156 polymorphic loci of 51 genes, and 68 polymorphisms were actually determined (Tables II and III). No heterozygosity was detected at the other 88 loci. The patients who are homozygous for one allele are shown as 1/1, those who are homozygous for the other allele are shown as 2/2, and the heterozygotes are shown as 1/2 (Table II).

The results of the statistical analysis for 66 polymorphisms and 2 null genotypes of *GSTT1* and *GSTM1* are summarized in Tables II and III, respectively. The genotype distributions of *CYP2E1* A10458T, *IRS4* His879Asp, *MAOB* Pro487Pro, and *GLUT2* Thr110Ile loci deviated significantly from Hardy-Weinberg equilibrium in the control group (Table II). In addition, the genotype distributions of the case group at loci *GPX3* (+92C/T), *TNF*α (−857C/T), *PPARγ2* His477His, and *TNFR* Met196Arg significantly deviated from the expected values calculated from the observed allele frequencies of the control group. The polymorphisms at these loci were not used for further analysis.

Combined null genotypes of GSTT1 and GSTM1. The genetic polymorphism of *GSTT1* and *GSTM1* is unique in that they are alternatively expressed as active (wild) or inactive (null genotype) forms. Among the 25 case patients, 15 (60.0%) were *GSTT1* null genotype and 17 (68.0%) were *GSTM1* null genotype, whereas among the 85 control patients, 33 (38.8%) were *GSTT1* null genotype and 40 (47.1%) were *GSTM1* null genotype (Table III). Thus the frequencies of null genotypes of both *GSTT1* and *GSTM1* were higher in the case group than in the control group, although such tendencies were not statistically significant ($P = .061$ and $P = .065$, respectively) (Table III).

The effect of the combined genotype of *GSTT1* and *GSTM1* on troglitazone-associated abnormal increases in liver enzyme levels was examined (Table IV). In the case group, the numbers of patients with the combined *GSTT1* wild genotype and *GSTM1* wild genotype (hereafter designated as wild/wild, with the same rule applicable to the genotype symbols that follow), wild/null, null/wild, and null/null were 3 (12%), 7 (28%), 5 (20%), and 10 (40%), respectively, and the corresponding numbers in the control group were 25 (29%), 27 (32%), 20 (24%), and 13 (15%), respectively. The distribution of the 4 genotypes in the case group was

Table II. Distributions of allele and genotype frequency in troglitazone-administered Japanese patients with type 2 diabetes

			Genotype frequency (observed)†				*Allele frequency*		*χ^2 Analysis (P value)*			
Gene	*Locus*	*Hepato-toxicity**	*1/1*	*1/2*	*2/2*	*Total*	*p(1)*	*q(2)*	*Hardy-Weinberg equilibrium (df = 1)*	*Genotype frequency difference (df = 2)*	*Allele frequency difference (df = 1)*	*Deviation from Hardy-Weinberg equilibrium (df = 1)*
First subset												
CYP2C9	A183C in exon 7	−	81	4	0	85	0.976	0.024	.824	.269	.274	.269
		+	25	0	0	25	1.000	0.000	ND			
CYP2C19	m2(G→A) at 636 in exon 4	−	68	16	1	85	0.894	0.106	.957	.532	.297	.123
		+	18	6	1	25	0.840	0.160	.592			
CYP2C19	m1(G→A) at 681 in exon 5	−	44	31	10	85	0.700	0.300	.225	.942	.787	.597
		+	12	10	3	25	0.680	0.320	.686			
CYP2E1	A10458T	−	50	29	6	85	0.759	0.241	.531	ND	ND	ND
		+	15	6	4	25	0.720	0.280	.043			
UGT1A1	TA repeats (6/7)	−	68	17	0	85	0.900	0.100	.306	1.000	1.000	.579
		+	20	5	0	25	0.900	0.100	.579			
UGT1A1	Gly71Arg in exon 1	−	53	30	2	85	0.800	0.200	.343	.556	.338	.241
		+	18	7	0	25	0.860	0.140	.416			
GLUT1	T→G (*XbaI*) in intron 2	−	5	27	53	85	0.218	0.782	.535	.883	.972	.803
		+	1	9	15	25	0.220	0.780	.807			
UCP1	A→C at −113 in 5'-region	−	71	13	1	85	0.912	0.088	.648	.546	.262	.229
		+	23	2	0	25	0.960	0.040	.835			
UCP1	A→C at −180 in 5'-region	−	71	13	1	85	0.912	0.088	.648	.546	.262	.229
		+	23	2	0	25	0.960	0.040	.835			
UCP1	Ala64Thr in exon 2	−	70	14	1	85	0.906	0.094	.753	.482	.220	.189
		+	23	2	0	25	0.960	0.040	.835			
UCP1	Met229Leu in exon 5	−	70	14	1	85	0.906	0.094	.753	.734	.450	.402
		+	22	3	0	25	0.940	0.060	.750			
ADRB3	Trp64Arg (TGG→CGG) in exon 1	−	57	25	3	85	0.818	0.182	.900	.617	.486	.328
		+	18	7	0	25	0.860	0.140	.416			
GPX1	Pro199Leu (CCC→CTC) in exon 2	−	72	13	0	85	0.924	0.076	.445	.350	.368	.332
		+	23	2	0	25	0.960	0.040	.835			

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Table II—Cont'd

			Genotype frequency (observed)†				*Allele frequency*		*χ^2 Analysis (P value)*			
Gene	*Locus*	*Hepato-toxicity**	*1/1*	*1/2*	*2/2*	*Total*	*p(1)*	*q(2)*	*Hardy-Weinberg equilibrium (df = 1)*	*Genotype frequency difference (df = 2)*	*Allele frequency difference (df = 1)*	*Deviation from Hardy-Weinberg equilibrium (df = 1)*
GPX3	+92 C→T in 3'-region	−	32	45	8	85	0.641	0.359	.165	ND	ND	.039
		+	15	9	1	25	0.780	0.220	.807			
GPX3	+97 C→T in 3'-region	−	84	1	0	85	0.994	0.006	.956	.353	.355	.188
		+	24	1	0	25	0.980	0.020	.919			
*TNF*α	Position −1031T→C in 5'-region	−	65	18	2	85	0.871	0.129	.579	.902	.846	.349
		+	19	5	1	25	0.860	0.140	.397			
*TNF*α	position −863 C→A in 5'-region	−	67	17	1	85	0.888	0.112	.946	.648	.587	.214
		+	19	5	1	25	0.860	0.140	.397			
*TNF*α	position −857 C→T in 5'-region	−	53	31	1	85	0.806	0.194	.127	ND	ND	.019
		+	12	13	0	25	0.740	0.260	.079			
*TNF*α	position −308 G→A in 5'-region	−	83	2	0	85	0.988	0.012	.913	.657	.659	.576
		+	24	1	0	25	0.980	0.020	.919			
*TNF*α	position −238 G→A in 5'-region	−	82	3	0	85	0.982	0.018	.868	.341	.344	.341
		+	25	0	0	25	1.000	0.000	ND			
*PPAR*γ*2*	Pro12Ala (CCA→GCA) in exon 1	−	78	7	0	85	0.959	0.041	.692	.565	.574	.432
		+	22	3	0	25	0.940	0.060	.750			
*PPAR*γ*2*	His477His (CAC→CAT) in exon 6	−	61	23	1	85	0.853	0.147	.469	ND	ND	.007
		+	13	12	0	25	0.760	0.240	.114			
Catalase	5'-region 1	−	38	42	5	85	0.694	0.306	.131	.930	.849	.571
		+	11	12	2	25	0.680	0.320	.607			
Catalase	5'-region 2	−	35	43	7	85	0.665	0.335	.214	.992	.951	.423
		+	10	13	2	25	0.660	0.340	.428			
Catalase	Asp388Asp (GAT→GAC)	−	19	46	20	85	0.494	0.506	.447	.451	.241	.142
		+	3	14	8	25	0.400	0.600	.405			
Catalase	Leu418Leu C→T in exon 10	−	80	5	0	85	0.971	0.029	.780	.716	.719	.691
		+	24	1	0	25	0.980	0.020	.919			

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Table II—Cont'd

			Genotype frequency (observed)†				*Allele frequency*		*χ^2 Analysis (P value)*			
Gene	*Locus*	*Hepato-toxicity**	*1/1*	*1/2*	*2/2*	*Total*	*p(1)*	*q(2)*	*Hardy-Weinberg equilibrium (df = 1)*	*Genotype frequency difference (df = 2)*	*Allele frequency difference (df = 1)*	*Deviation from Hardy-Weinberg equilibrium (df = 1)*
Catalase	3-region	−	40	40	5	85	0.706	0.294	.219	.936	.847	.344
		+	12	12	1	25	0.720	0.280	.341			
DT-diaphorase	Arg139Trp (CGG→TGG) in exon 4	−	77	7	1	85	0.947	0.053	.099	.861	.712	.665
		+	23	2	0	25	0.960	0.040	.835			
DT-diaphorase	Pro187Ser (CCT→TCT) in exon 6	−	35	36	14	85	0.624	0.376	.367	.820	.832	.755
		+	10	12	3	25	0.640	0.360	.835			
Second subset												
IRS1	Gly971Arg (GGG→AGG)	−	74	6	0	80	0.963	0.038	.727	.597	.603	.573
		+	22	1	0	23	0.978	0.022	.915			
IRS1	Ala804Ala (GCA→GCG)	−	40	34	6	80	0.713	0.288	.738	.653	.432	.250
		+	9	12	2	23	0.652	0.348	.472			
IRS1	Met209Thr (ATG→ACG)	−	79	1	0	80	0.994	0.006	.955	.590	.591	.590
		+	23	0	0	23	1.000	0.000	ND			
IRS1	6 bp deletion at 682	−	78	2	0	80	0.988	0.013	.910	.444	.446	.444
		+	23	0	0	23	1.000	0.000	ND			
IRS1	AGC repeat (7/8)	−	77	3	0	80	0.981	0.019	.864	.896	.897	.848
		+	22	1	0	23	0.978	0.022	.915			
IRS4	His879Asp (CAT→GAT)	−	18	14	48	80	0.313	0.688	.000	ND	ND	ND
		+	2	9	12	23	0.283	0.717	.867			
LEP	−2548G→A in 5'-region	−	8	25	47	80	0.256	0.744	.107	.605	.814	.667
		+	1	9	13	23	0.239	0.761	.718			
LEPR	Lys109Arg (AAG→AGG) in exon 4	−	3	22	55	80	0.175	0.825	.670	.543	.986	.315
		+	0	8	15	23	0.174	0.826	.313			
LEPR	Gln223Arg (CAG→CGG) in exon 6	−	0	17	63	80	0.106	0.894	.288	.686	.703	.576
		+	0	4	19	23	0.087	0.913	.648			
LEPR	Ser343Ser (AGT→AGC) in exon 9	−	70	9	1	80	0.931	0.069	.277	.185	.180	.054
		+	17	6	0	23	0.870	0.130	.472			

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Table II—Cont'd

Gene	Locus	Hepato-toxicity*	Genotype frequency (observed)†				Allele frequency		χ^2 Analysis (P value)			
			1/1	1/2	2/2	Total	p(1)	q(2)	Hardy-Weinberg equilibrium (df = 1)	Genotype frequency difference (df = 2)	Allele frequency difference (df = 1)	Deviation from Hardy-Weinberg equilibrium (df = 1)
LEPR	Lys656Asn (AAG→AAC) in exon 14	−	66	14	0	80	0.913	0.088	.391	.031	.038	.032
		+	23	0	0	23	1.000	0.000	ND			
LEPR	Pro1019Pro (CCG→CCA) in exon 20	−	0	16	64	80	0.100	0.900	.320	.290	.322	.108
		+	0	7	16	23	0.152	0.848	.389			
LEPR	CTTTA deletion in 3'-region	−	0	16	64	80	0.100	0.900	.320	.448	.472	.422
		+	0	3	20	23	0.065	0.935	.738			
CD36	Pro90Ser (CCT→TCT) in exon 4	−	76	4	0	80	0.975	0.025	.819	.505	.511	.391
		+	21	2	0	23	0.957	0.043	.827			
CD36	G/A in 3'-region (exon 15)	−	17	39	24	80	0.456	0.544	.876	.723	.600	.400
		+	5	13	5	23	0.500	0.500	.532			
HGF	C→T in intron 11	−	60	20	0	80	0.875	0.125	.201	.916	.922	.451
		+	17	6	0	23	0.870	0.130	.472			
HGF	A→T in intron 17	−	60	20	0	80	0.875	0.125	.201	.916	.922	.451
		+	17	6	0	23	0.870	0.130	.472			
HGF	A→C in intron 17	−	60	20	0	80	0.875	0.125	.201	.916	.922	.451
		+	17	6	0	23	0.870	0.130	.472			
Fas antigen	−670GA→GG in 5'-region	−	23	35	22	80	0.506	0.494	.264	.108	.334	.066
		+	6	15	2	23	0.587	0.413	.098			
Fas antigen	−1377CG→CA in 5'-region	−	30	35	15	80	0.594	0.406	.405	.416	.654	.278
		+	8	13	2	23	0.630	0.370	.307			
CTLA4	Thr17Ala (ACC→GCC) in exon 1	−	16	31	33	80	0.394	0.606	.092	.913	.976	.675
		+	4	10	9	23	0.391	0.609	.675			
SEP	+14G→A in 3'-region	−	42	27	11	80	0.694	0.306	.066	.447	.798	.604
		+	10	11	2	23	0.674	0.326	.672			

Continued on next page

Table II—Cont'd

			Genotype frequency (observed)†				*Allele frequency*		*χ^2 Analysis (P value)*			
Gene	*Locus*	*Hepato-toxicity**	*1/1*	*1/2*	*2/2*	*Total*	*p(1)*	*q(2)*	*Hardy-Weinberg equilibrium (df = 1)*	*Genotype frequency difference (df = 2)*	*Allele frequency difference (df = 1)*	*Deviation from Hardy-Weinberg equilibrium (df = 1)*
TNFR2	Met196Arg (ATG→AGG) in exon 6	−	61	17	2	80	0.869	0.131	.542	ND	ND	.009
		+	15	6	2	23	0.783	0.217	.263			
p22 phax	+24A→G in 3'-region	−	18	47	15	80	0.519	0.481	.114	.610	.457	.098
		+	3	15	5	23	0.457	0.543	.132			
NOS3	−922A→G in 5'-region	−	64	15	1	80	0.894	0.106	.909	.656	.646	.324
		+	17	6	0	23	0.870	0.130	.472			
MAOB	Pro487Pro (CCC→CCT) in exon 15	−	54	17	9	80	0.781	0.219	.001	ND	ND	ND
		+	17	5	1	23	0.848	0.152	.450			
RDH5	Val200Val (GTC→GTG) in exon 4	−	78	2	0	80	0.988	0.013	.910	.642	.645	.559
		+	22	1	0	23	0.978	0.022	.915			
TPMT	Tyr180Phe (TAT→TTT) in exon 8	−	80	0	0	80	1.000	0.000	ND	.061	.062	ND
		+	22	1	0	23	0.978	0.022	.915			
TPMT	Tyr240Cys (TAT→TGT) in exon 10	−	75	5	0	80	0.969	0.031	.773	.681	.687	.582
		+	21	2	0	23	0.957	0.043	.827			
GLUT2	Val101Ile (GTA→ATA) in exon 3	−	75	5	0	80	0.969	0.031	.773	.731	.735	.707
		+	22	1	0	23	0.978	0.022	.915			
GLUT2	Thr110Ile (ACT→ATT) in exon 3	−	76	3	1	80	0.969	0.031	.001	ND	ND	ND
		+	22	1	0	23	0.978	0.022	.915			
GLUT2	Gly519Glu (GGA→GAA) in exon 10	−	77	3	0	80	0.981	0.019	.864	.346	.350	.346
		+	23	0	0	23	1.000	0.000	ND			
LPL	*Hin*dIII (T→G) in intron 8	−	53	23	4	80	0.806	0.194	.476	.258	.089	.066
		+	19	4	0	23	0.913	0.087	.648			

Continued on next page

Table II—Cont'd

			Genotype frequency (observed)†				*Allele frequency*		*χ^2 Analysis (P value)*			
Gene	*Locus*	*Hepato-toxicity**	*1/1*	*1/2*	*2/2*	*Total*	*p(1)*	*q(2)*	*Hardy-Weinberg equilibrium (df = 1)*	*Genotype frequency difference (df = 2)*	*Allele frequency difference (df = 1)*	*Deviation from Hardy-Weinberg equilibrium (df = 1)*
LPL	*Pvu*II (C→T) in intron 6	−	46	27	7	80	0.744	0.256	.305	.288	.590	.194
		+	13	10	0	23	0.783	0.217	.183			
LPL	Thr388Thr (ACA→ACC) in exon 8	−	0	9	71	80	0.056	0.944	.594	.324	.337	.309
		+	0	1	22	23	0.022	0.978	.915			
APOA1	+134C/T in intron 2	−	29	36	15	80	0.588	0.413	.522	.817	.601	.550
		+	9	11	3	23	0.630	0.370	.899			
APOC3	Thr74Ala (ACT→GCT)	−	79	1	0	80	0.994	0.006	.955	.590	.591	.590
		+	23	0	0	23	1.000	0.000	ND			

df, Degrees of freedom; ND, not determined. Genes and their corresponding substances are as follows: *UGT1A1*, uridine diphosphate–glucuronosyltransferase 1A1; *GLUT*, glucose transporter; *UCP*, uncoupling protein; *ADRB3*, β_3-adrenergic receptor; *GPX*, glutathione peroxidase; *TNF*, tumor necrosis factor; *DT-diaphorase*, nicotinamide adenine dinucleotide phosphate, reduced:quinone acceptor oxidoreductase; *PPAR*, peroxisome proliferator–activated receptor; *IRS*, insulin receptor substrate; *LEP*, leptin; *LEPR*, leptin receptor; *HGF*, hepatocyte growth factor; *CTLA4*, cytotoxic T-lymphocyte–associated molecule 4; *SEP*, selenoprotein P; *TNFR*, tumor necrosis factor receptor; *NOS*, nitric oxide synthase; *MAOB*, monoamine oxidase B; *RDH5*, retinol dehydrogenase 5; *TPMT*, thiopurine methyltransferase; *LPL*, lipoprotein lipase.

*−, Control patients; +, case patients.

†The patients who are homozygous for one allele are shown as 1/1, those who are homozygous for the other allele are shown as 2/2, and the heterozygotes are shown as 1/2.

significantly different from that in the control group ($P = .043$) (Table IV). These data indicated that the combined *GSTT1* and *GSTM1* null genotype seems to cooperatively enhance the susceptibility to troglitazone-associated transaminase elevations.

To evaluate the risk of the null/null genotype, we compared the frequency of the null/null genotype in the case and control groups with that of the other 3 combined genotypes (Table IV). These data indicate that the presence of the combined *GSTT1* and *GSTM1* null genotype is a risk factor for enhanced susceptibility to transaminase elevations associated with troglitazone (OR, 3.692; 95% CI, 1.354-10.066; $P = .008$).

LEPR Lys656Asn. Among the other 60 polymorphic loci, only *LEPR* Lys656Asn showed a significant difference in both genotype and allele frequencies between the case and control groups ($P = .031$ and $P = .038$, respectively) (Table II). The genotype at this gene locus was 1/1 (G/G) in all 23 case patients, whereas in the control patients, 66 of 80 possessed the 1/1 (G/G) genotype (82.5%) and the remainder had the 1/2 (G/C) genotype (17.5%). The patients with type 2 diabetes who had the 1/2 (G/C) genotype tended to be slightly resistant to troglitazone-associated abnormal increases in liver enzyme levels. ORs could not be calculated because the case patients had the same genotype.

DISCUSSION

In this study we analyzed genetic polymorphisms of selected genes (candidate gene approach) to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with type 2 diabetes mellitus. We selected 51 different genes related to drug metabolism, apoptosis, production and elimination of reactive oxygen species, and signal transduction pathways of PPARγ2 and insulin as candidates for analysis. To improve the statistical analysis, we made efforts to recruit as many Japanese patients for the case group as possible. However, because of the rarity of troglitazone-associated abnormal increases in liver enzyme levels observed clinically, it was not feasible to have equal numbers of case and control patients. Consequently, we recruited a total of 110 patients in the first subset of the genotyping study and

Table III. *GSTT1* and *GSTM1* polymorphisms and troglitazone-associated hepatotoxicity in case and control patients with type 2 diabetes (n = 110)

		No. of patients			*Probability*	
Factor	*Group*	*Total*	*Wild*	*Null*	χ^2 *Value*	*P value*
GSTT1	Control	85	52	33	3.522	.061
	Case	25	10	15		
GSTM1	Control	85	45	40	3.393	.065
	Case	25	8	17		

GSTT1, Gene for glutathione-*S*-transferase theta 1; *GSTM1,* glutathione-*S*-transferase mu 1.

Table IV. Distribution of combined *GSTT1* and *GSTM1* genotype in case and control patients with type 2 diabetes (n = 110)

	GSTT1/GSTM1 genotype [No. of patients (%)]					*Probability*		*Odds*	
Patients	*Wild/wild*	*Wild/null*	*Null/wild*	*Null/null*	*Total*	χ^2 *Value*	*P value*	*ratio*	*95% Confidence interval*
Control	25 (29)	27 (32)	20 (24)	13 (15)	85 (100)	7.130	.008*	1.000	1.354-10.066
Case	3 (12)	7 (28)	5 (20)	10 (40)	25 (100)			3.692	

*The frequency of the null/null genotype in the case and control groups was compared with that of the other 3 combined genotypes, including wild/wild, wild/null, and null/wild. The data indicate that the presence of the combined *GSTT1* and *GSTM1* null genotype is a risk factor for enhanced susceptibility to transaminase elevations.

103 patients in the second subset. This sample size was estimated to be sufficient to achieve a statistical power of 80% to detect an OR of 4.5 when 15% of the Japanese population had a genotype mutation.

We performed the association study to survey susceptible genetic factors for troglitazone-associated hepatotoxicity. Because the number of patients recruited in this study was limited, we took a direct sequencing strategy to raise the accuracy of genotyping. We selected the candidate genes potentially involved in troglitazone-associated hepatotoxicity, sequenced a number of polymorphic sites simultaneously, determined the actual polymorphic loci, and identified the association of the combined null genotype of *GSTT1* and *GSTM1* and the *LEPR* Lys656Asn genotype with abnormal increases in liver enzyme levels in a Japanese population with type 2 diabetes.

We have demonstrated that the combined null genotype of *GSTT1* and *GSTM1* might be a susceptible genetic factor for troglitazone-associated abnormal increases in liver enzyme levels. GSTs comprise a supergene family of enzymes including alpha, mu, pi, and theta[30] and catalyze the detoxification of a variety of reactive toxic compounds, chemicals, and their metabolites. It is therefore reasonable to speculate that homozygosity for null alleles is associated with a toxic effect of chemicals. In most patients treated with troglitazone, the onset of hepatotoxicity is late and the peak values of liver enzyme elevations occurred clinically between 2 and 5 months after the start of troglitazone medication.[13] In troglitazone-treated patients with type 2 diabetes having this combined null genotype, therefore, the putative reactive metabolites may gradually accumulate in the liver and form covalent adducts to hepatic proteins, leading to modulation of liver function in a deleterious fashion.

The troglitazone-associated transaminase elevations might be due to the combined null genotype of *GSTT1* and *GSTM1* in patients treated with troglitazone. However, the expected ratio to find the patients with the *GSTT1* and *GSTM1* double-null genotype among those who exhibited troglitazone-associated hepatotoxicity is in the range of 0.21 to 0.59 (95% CI). The combined null genotype of *GSTT1* and *GSTM1* may, therefore, only partially account for abnormal increases in liver enzyme levels with troglitazone therapy. There are supposed to be other genetic factors responsible for the hepatotoxicity.

Recently, several studies have reported the possibility of chemically reactive intermediates potentially involved in the hepatotoxicity associated with troglitazone. Enzymes involved in the production and elimination of these metabolites are supposed to be susceptible genetic factors for troglitazone-associated hepatotoxicity. Troglitazone has been reported to be a potential inducer of *CYP3A4* in human hepatocytes, indicating the autoinduction of formation of the reactive metabolites.[31] Kassahun et al[32] have reported that troglitazone was metabolized to 5 glutathione (GSH) conjugates when incubated with human liver microsomes and with complementary DNA–expressed CYP in the presence of GSH, but GSTs do not seem to be

involved in this pathway. Tettey et al[33] have shown that GSH adducts of troglitazone are produced in vivo in rats and this reaction is enhanced by CYP3A, indicating the involvement of CYP and GSTs in the metabolism of troglitazone. However, there are no reports that rats showed the liver failure seen in humans even when exposed to much higher quantities of troglitazone. Yamamoto et al[34] have identified an epoxide of a quinone metabolite of troglitazone catalyzed by CYP3A4. This epoxide may be eliminated by GSTs and epoxide hydrolase.[34] Recently, *CYP3A5* has been reported to be polymorphically expressed at high levels.[35] CYP3As[31-33,35] and their modulators (such as pregnane X receptor), epoxide hydrolase, other family members of GSTs, and so on could be the potential susceptible genetic factors for the hepatotoxicity associated with troglitazone. The variable expression of these proteins in the metabolism of troglitazone in the absence of *GSTT1* and *GSTM1* functions might cooperatively modulate the susceptibility to and represent an individual risk for troglitazone-associated hepatotoxicity.

During the course of this study, Simon et al[36] reported a similar association study regarding hepatotoxicity observed in patients treated with tacrine, a drug for treatment of Alzheimer's disease. They primarily examined whether the *GSTT1* null genotype and the *GSTM1* null genotype predict individual susceptibility to transaminase elevations associated with tacrine and concluded that either of these null genotypes alone was not a risk factor for development of hepatotoxicity but that the combined null genotype of *GSTT1* and *GSTM1* was a risk factor. On the other hand, the *GST* null genotype has been reported to be associated with various malignancies of cancer and inflammatory bowel disorders,[37] which might be caused by a deficiency in detoxification of reactive oxygen species, potential mutagens, and carcinogens. Taken together, although the mechanisms of drug-induced hepatotoxicity and association with some diseases are unclear, the property of GSTs in the detoxification and inactivation against chemical exposure may be extensively involved not only in antitoxic effects but also in antitumor and anti-inflammatory effects. It would be of interest from the toxicologic and epidemiologic points of view to determine the significance of the combination of *GSTT1* null and *GSTM1* null genotypes in a wide variety of drug-induced toxicity and disease susceptibility.

In this study an association with transaminase elevations was found in the polymorphism of *LEPR* Lys656Asn (Table II). However, because no association of *LEPR* Lys656Asn with obesity is reported,[38] the amino acid substitution from Lys656 to Asn656 does not appear to affect the receptor activity. In addition, no articles reporting the relationship of this genetic polymorphism to diabetes or drug response are available. The significance of this polymorphism with abnormal increases in liver enzyme levels is, therefore, unclear at the moment. This finding could be due to chance, in view of the many statistical comparisons that were made.

In conclusion, we report here that the combined *GSTT1* and *GSTM1* null genotype and the *LEPR* Lys656Asn genotype might be susceptible genetic factors for troglitazone-associated abnormal increases in liver enzyme levels in Japanese patients with type 2 diabetes. Several issues still remain unclear, as follows: whether the current association study can be experimentally verified by cellular and animal experiments; whether there are reactive metabolites, which are supposed to be detoxified by GSTs; whether this study is applicable to other ethnic groups; determination of other genetic factors involved in the hepatotoxicity; and the involvement of the *LEPR* Lys656Asn genotype in transaminase elevations. This study has provided the first report describing the association of genetic polymorphisms with troglitazone-associated hepatotoxicity; further studies are therefore necessary to clarify the complete mechanisms for troglitazone-associated hepatotoxicity.

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Appendix Table I. Polymorphisms analyzed and polymerase chain reaction primers used in this study

No.	*Gene*	*Polymorphism*	*Forward primer*	*Reverse primer*
1	*ADRB3*	Trp64Arg (TGG→CGG) in exon 1	GGCCTCACGAGAACAGC	ACACAGCACGTCCACCGA
2	*ALB*	Arg-2His (CGT→CAT) in exon 1	TTTTCTCTTCTGTCAACCC	AAATAAATGCCAAAATAATTCT
3	*APOA1*	+134C/T in intron 2	CAGCGGCAGAGACTATGT	GGAAAGGGGCTTGCTAC
4	*APOA1*	Gly26Arg (GGC→CGC)	ACAGCTGGCCTGATCTG	GATCCCATTCCGGTTTC
5	*APOA1*	Leu224Leu (CTG→TTG)	CCCCCTACAGCGACGAG	ATTCTGAGCACCGGGAAG
6	*APOC3*	Thr74Ala (ACT→GCT)	GGACATGGGTGTGGGTC	AGGCATGAGGTGGGGTAG
7	*APOC3*	Lys58Glu (AAG→GAG)	GGACATGGGTGTGGGTC	AGGCATGAGGTGGGGTAG
8	*CASP9*	+609A→T in 3'-region	CACTGGTCTGTAGGGAT	AAAGTCTCTTTTGTAAGGAA
9	*Catalase*	5'-region 1	TAGCGCCGAGCAGCCAATCAGAAG	TGTCAGCCATAGCGTGCGGTTTGC
10	*Catalase*	5'-region 2	CTGATTGGCTGAGCCTGAAGTCGC	TCGGGGAGCACAGAGTGTACCTGC
11	*Catalase*	Asp388Asp (GAT→GAC)	GCCTATCCTGACACTCACCGCCAT	ATCTGCTCCACGTGCCCTCTGCC
12	*Catalase*	Leu418Leu C→T in exon 10	TCTTATTCCTAAGTGCATCTGGGTGG	GCAGGTGACTTCCATAGCAGATAAAG
13	*Catalase*	3'-region	TCAGCACTGATTTCACAACAGATCAA	CATTTCCATTTTTAAGGGTAAACATCTG
14	*Catalase*	Exon 9	GCCTATCCTGACACTCACCGCCAT	ATCTGCTCCACGTGCCCTCTGCC
15	*CD36*	Pro90Ser (CCT→TCT) in exon 4	TGCTTTTAAAAATTGGGTTA	TTTTTGGCAAGAAAATAATAAG
16	*CD36*	G/A in 3'-region in exon 15	TGCAACTTACGCTTGG	TAAAGAAATGTGGATTCAGAT
17	*CD36*	Ala→Pro (GCA→CCA) in exon 12	GTTTCAATTAGTCCTGTTTAAC	TTTCCACTTACTTACATAGATTT
18	*CD36*	1 bp (A) deletion at 30 in exon 12	GTTTCAATTAGTCCTGTTTAAC	TTTCCACTTACTTACATAGATTT
19	*CD36*	Val154Phe (GTT→TTT) in exon 6	TGACTTTGTTTTTGTAGGCT	GCCATTCATATTTGGTACTT
20	*CD36*	Pro191Pro (CCG→CCA) in exon 6	TGACTTTGTTTTTGTAGGCT	GCCATTCATATTTGGTACTT
21	*CTLA4*	Thr17Ala (ACC→GCC) in exon 1	ATCCTGAAAGGTTTTGCTCTAC	ATAAATGACTGCCCTTGACTG

Continued on next page

Appendix Table I—Cont'd

No.	*Gene*	*Polymorphism*	*Forward primer*	*Reverse primer*
22	*CYP1A1*	G3320A	AGTATTGCCTCAGTTTCCTTTC	TCCCAAACCACATTGCTTTC
23	*CYP2C19*	m2 (G→A) at 636 in exon 4	CTCCCTGCAATGTGATCTGC	ACTTCAGGGCTTGGTCAATA
24	*CYP2C19*	m1 (G→A) at 681 in exon 5	CCAGAGCTTGGCATATTGTATC	GCAATCAATAAAGTCCCGAGG
25	*CYP2C9*	A183C in exon 7	GAATTGCTACAACAAATGTGCC	TTGGGACTTCGAAAACATGG
26	*CYP2E1*	A10458T	TGTAGCTTCTACCTATTGGG	GACAGGGTTTCATCATGTT
27	*CYP3A4*	A→G in 5'-region	AGGCTCTGTCTGTCTGGGTT	GGAAGTTGGCAAAGAATCAC
28	*CYP3A4*	Ser222Pro (TCA→CCA) in exon 7	CATCTTTCTCCACTCAGCGTC	TGACAGGGTTTGTGACAGGG
29	*CYP3A4*	Met445Thr (ATG→ACG) in exon 12	TCTCATCTCAACAAGACTGAAAG	GGTTTGAAGGAGAAGTTCTGAAG
30	*DT-diaphorase*	Arg139Trp (CGG→TGG) in exon 4	GAGCCACCTTCTGGGCTTG	CAAACAAACACCCCTGCATCA
31	*DT-diaphorase*	Pro187Ser (CCT→TCT) in exon 6	GTTTGACTTACCTCTCTGTGCTTTCTG	GTTTAGGTCAAAGAGGCTGCTTGGAGC
32	*Fas antigen*	–670GA→GG in 5'-region	GCCAGGAAATAATGAGTAACGAA	CTAAAGGCTTCTGCTGTAGTTCAA
33	*Fas antigen*	–1377CG→CA in 5'-region	CCTTTCCTTCCCTCACACCC	TCCTTCAGGCTTCCTCTCAGTTGAC
34	*GLUT1*	T→G (*Xba*I) in intron 2	GCTCACAGACCCTACCTAATG	ATAAAGCTAGTCTCCAGACCC
35	*GLUT2*	Val101lle (GTA→ATA) in exon 3	CCGAAAAGCTATCAACAACTATGTTA	AACAACTCTAAAGCTATTCCACAAGAA
36	*GLUT2*	Thr110lle (ACT→ATT) in exon 3	AACTGCCCACAATCTCATACTCAAT	AACAATTAACCAAACTGTCGCAACA
37	*GLUT2*	Gly519Glu (GGA→GAA) in exon 10	CAGGACTTCTGTGGACCTTATGTGT	TTGATGACATTTCTGATGAGAGCAC
38	*GLUT2*	Pro68Leu (CCC→CTC) in exon 3	CATAGAATGTTTGAAATAGTAAAGC	TAGGAAAATGAAAATATGAAAGTT
39	*GPX1*	Pro199Leu (CCC→CTC) in exon 2	GGTCTCCGGTGTGTCGC	ATCAGGTGTTCCTCCCTCGT
40	*GPX3*	+92 C→T in 3'-region	GGGTCAAGAGGAAGTAACTG	CACAATCACGCATACCTG
41	*GPX3*	+97 C→T in 3'-region	GGGTCAAGAGGAAGTAACTG	CACAATCACGCATACCTG
42	*GPX4*	C→T in 3'-region	CCCGACAGGTGATAGAGA	CAGCTGACGGCAACTG
43	*GSTM1*	Null	GAACTCCCTGAAAAGCTAAAGC	GTTGGGCTCAAATATACGGTGG
44	*GSTT1*	Null	TTCCTTACTGGTCCTCACATCTC	TCACCGGATCATGGCCAGCA

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Appendix Table I—Cont'd

No.	*Gene*	*Polymorphism*	*Forward primer*	*Reverse primer*
45	*GYS1*	Lys130Glu (AAG→GAG) in exon 3	CCAGAGCCTCCCTATGGT	TGTCAGACGGGGCCTAC
46	*GYS1*	Gly140Gly (GGA→GGG) in exon 3	CCAGAGCCTCCCTATGGT	TGTCAGACGGGGCCTAC
47	*GYS1*	*Xba*I (C→T) in intron 14	CCTAACAGGAAGGATCGCAT	CAGTTTCTGAGCCATACCCC
48	*GYS1*	Asp671Asp (GAC→GAT) in exon 16	CCACATCGCCGTATGCAG	GGTGGGGCGGACTTAGTTAC
49	*GYS1*	Pro691Ala (CCA→GCA) in exon 16	CCACATCGCCGTATGCAG	GGTGGGGCGGACTTAGTTAC
50	*GYS2*	Asn39Ser (AAT→AGT) in exon 1	TTCTCCTGGGATTACAACTA	TCCCTCTTCAACACATACAC
51	*GYS2*	His446Asp (CAC→GAC) in exon 11	AGTCATCATCATTAGTGCCA	AAGTTTAAAGTTCGCCACAT
52	*GYS2*	Ala339Pro (GCT→CCT) in exon 7	CAAGATTTTGTTCGAGGTCATT	CTGAAGCCATTTTTAGAATACGATT
53	*HGF*	C→T in intron 11	TGACTGAACTCTATGCCATC	ATTATGCTTTGTTGCTTGAA
54	*HGF*	A→T in intron 17	GCATATATTCATTTATGGTTTT	AGCAGTAACATCATATTAGTGA
55	*HGF*	A→C in intron 17	GCATATATTCATTTATGGTTTT	AGCAGTAACATCATATTTAGTGA
56	*HGF*	3370C→A in intron 4	TTTGTTAGTGTCCCAATGTA	AAAGTATATTTGGATGTAACCC
57	*HGF*	Ser153lle (AGT→ATT) in exon 4	GCATATGTTTTGCATAGTTG	TAAACCTTGCCGTAATACA
58	*HGF*	T→G in intron 11	AGGAAATACTTTTATAAGTTCG	TTGCCTTTAATGCTAGAAT
59	*IGF1*	Ala187Asp (GCT→GAT) in exon 4	AGTATCAGCCCCCATCTACCA	TAGCTCCAGCAGGCCTACTTTT
60	*IGF2*	A→G in 5'-region	GCCAGCAATCGGAAGTG	GGGGGAAGGGGGTTAGTT
61	*IGF2*	G→A in 3'-region	CCCCTCCTTTGGTCTTACTG	AACACCCCACAAAAGCTCA
62	*IGFR2*	Gly1449Val (GGC→GTC) in exon 31	AGCCTGGGGAGTCACTAAAG	TGGTAAGGGCGCATCAT
63	*IGFR2*	Gly1464Glu (GGG→GAG) in exon 31	AGCCTGGGGAGTCACTAAAG	TGGTAAGGGCGCATCAT
64	*IR*	Val985Met (GTG→ATG) in exon 17	TCCTGGATCACAGAACTCAT	GACTCGTTGACCGTCTTC
65	*IRS1*	Gly971Arg (GGG→AGG)	CTTCTGTCAGGTGTCCATCC	TGGCGAGGTGTCCACGTAGC

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Appendix Table I—Cont'd

No.	*Gene*	*Polymorphism*	*Forward primer*	*Reverse primer*
66	*IRS1*	Ala804Ala (GCA→GCG)	GCCAAGATCCTTTAAGCA	GCTGTGTCCACCTTTCG
67	*IRS1*	Met209Thr (ATG→ACG)	TGGGGAGGACTTGAGCTA	ACGCTGATGGGGTTAGAG
68	*IRS1*	6–base pair deletion at 682	ATCCCATCAGACGCCATC	CTCAGGGCCGTAGTAGCAGT
69	*IRS1*	AGC repeat (7/8)	GCCCCATCTGCCTCGAA	CGGCCCCAGGTCCATC
70	*IRS1*	Ser809Phe (TCT→TTT)	GCCAAGATCCTTTAAGCA	GCTGTGTCCACCTTTCG
71	*IRS1*	Gly819Arg (GGA→AGA or CGA)	GCCAAGATCCTTTAAGCA	GCTGTGTCCACCTTTCG
72	*IRS1*	Met613Val (ATG→GTG)	CAGAGGAGGGTCTGGAAATG	GCCACCTCCAATGTCAGG
73	*IRS1*	Gly625Gly (GGC→GGT)	CAGAGGAGGGTCTGGAAATG	GCCACCTCCAATGTCAGG
74	*IRS1*	Leu142Leu (CTT→CTC)	GACGAGCACTTTGCCATC	GCTGCCTCCGAGTTCAG
75	*IRS1*	Pro170Arg (CCC→CGC)	GACGAGCACTTTGCCATC	GCTGCCTCCGAGTTCAG
76	*IRS1*	Ala512Pro (GCC→CCC) in exon 1	CGCGGTGAGGAGGAGCTAAG	TGAGGACTGGGACGGGTTCT
77	*IRS1*	Ser892Gly (AGC→GGC) in exon 1	GCCCCATCTGCCTCGAA	CGGCCCCAGGTCCATC
78	*IRS2*	Gly879Ser (GGC→AGC) in exon 1	ACAGCGACCAGTACGTG	GCCAAAGTCGATGTTGA
79	*IRS4*	His879Asp (CAT→GAT)	GTGAGTATGTGCCAATGTTACCTG	GAGTAGAGGGAGCTGGTGTATTGC
80	*IRS4*	Leu34Phe (CTT→TTT)	TCGCTCACCTTAAAACCATC	CTTCTGTTTCCGCAGGTAG
81	*IRS4*	Arg411Gly (CGA→GGA)	CAGAAGGTCCCGCTTTG	TGTAGTCACCTCCGCTTCC
82	*IRS4*	Lys883Thr (AAG→ACG)	GTGAGTATGTGCCAATGTTACCTG	GAGTAGAGGGAGCTGGTGTATTGC
83	*IRS4*	Gly584Cys (GGT→TGT)	GCTCCAGTAGCCATAGCTCG	GTGGCATTTGCTGTTGCTT
84	*LEP*	–2548G→A in 5'-region	GGCAAAATTGAGGATATTAC	ATTTGGGAACTGATAAGTGT
85	*LEP*	Thr48Thr (ACG→ACA) in exon 2	AACCCTGTGCGGATTCTT	TCTCAGCTTCCAGTAGGTGC
86	*LEP*	Glu126Gln (GAG→CAG)	GGGTGCAGGATACAAGGG	GCAAAAGGAGGCTTAGGGTC
87	*LEP*	1 bp deletion at 131 in exon 3	GGGTGCAGGATACAAGGG	GCAAAAGGAGGCTTAGGGTC
88	*LEP*	Arg105Trp (CGG→TGG)	GGGTGCAGGATACAAGGG	GCAAAAGGAGGCTTAGGGTC
89	*LEP*	Val110Met (GTG→ATG)	GGGTGCAGGATACAAGGG	GCAAAAGGAGGCTTAGGGTC

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Appendix Table I—Cont'd

No.	*Gene*	*Polymorphism*	*Forward primer*	*Reverse primer*
90	*LEP*	33C→T in 3'-region	GGGTGCAGGATACAAGGG	GCAAAAGGAGGCTTAGGGTC
91	*LEP*	–2437T→G in 5'-region	GGCAAAATTGAGGATATTAC	ATTTGGGAACTGATAAGTGT
92	*LEPR*	Lys109Arg (AAG→AGG) in exon 4	TCAAGTGGTACTCACTTTTCTAAC	AGACATCTATTTCATACAGGTATC
93	*LEPR*	Gln223Arg (CAG→CGG) in exon 6	ACCCTTTAAGCTGGGTGTCCCAAATAG	AGCTAGCAAATATTTTTGTAAGCAATT
94	*LEPR*	Ser343Ser (AGT→AGC) in exon 9	CTTACCAGAATGTTTGTCTTCATCTG	GCTAAATTCATCCACCAAACAATCTC
95	*LEPR*	Lys656Asn (AAG→AAC) in exon 14	GGCTGAAAAGTATTTCTTCAAAAAC	ACAGGATTATGGACCATGAAGTAC
96	*LEPR*	Pro1019Pro (CCG→CCA) in exon 20	CAGCAACTCTAAACCAAGTGAAACTG	GGTGTGGTGAAATTATGTTGGGATGC
97	*LEPR*	CTTTA deletion in 3'-region	GCCTCAATTCCAAACTTGT	CGTGTCTACATTGGTAGGCT
98	*LEPR*	Ser492Thr (AGT→ACT) in exon 11	GACTGCTGTTTTAAACAACAAATCAG	CTGCATACAAATCTGCTAACACAAATG
99	*LEPR*	Ala976Asp (GCC→GAC) in exon 20	CTGTTTGTATTAGTGACCAGTTCAAC	CCTTGTTCTTCACCAGTTTCACTTGG
100	*LEPR*	Gln270Pro (CAA→CCA) in exon 7	TTAAGTGAAGCCTGATCC	GTAACTAGATTTCCTATAGCC
101	*LEPR*	+20G/A in intron 7	TTAAGTGAAGCCTGATCC	GTAACTAGATTTCCTATAGCC
102	*LEPR*	Lys204Arg (AAA→AGA) in exon 6	TCAATATAGGCCTGAAGTGTTA	TGGGCTGAACTGACATTAG
103	*LPL*	*Hin*dIII (T→G) in intron 8	CTACCTGGATAATCAAAGATTCAA	GTGATACAAGCAAATGACTAAAGAG
104	*LPL*	*Pvu*II (C→T) in intron 6	TCAAGGCTCTGTCAGTGTCC	TGCTGCTTTAGACTCTTGTCC
105	*LPL*	Asn291Ser (AAT→AGT) in exon 6	ATCGACTCTCTGTTGAATGAAG	ACCAGTCATCATCTCTGTTCC
106	*LPL*	Thr388Thr (ACA→ACC) in exon 8	ACTAAATGCCATCGACCTTC	TCAGGTGGGGGTCTAAAGT
107	*LPL*	Gly188Glu (GGG→GAG)	AAATTTACAAATCTGTGTTCCT	TAATATTTACCTCCAAGTCCTC
108	*LPL*	Glu145Glu (GAG→GAA)	TTCAGTATTTCCTATATTTGGA	GACCAACGAAATTGCTT
109	*MAOB*	Pro487Pro (CCC→CCT) in exon 15	AGAGCTGGAGAAAGTACAGA	CAAGCACAAAGCCATAAT

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Appendix Table I—Cont'd

No.	*Gene*	*Polymorphism*	*Forward primer*	*Reverse primer*
110	*MAOB*	+220A→G in 3'-region	AGAGCTGGAGAAAGTACAGA	CAAGCACAAAGCCATAAT
111	*MRP2*	Position 1815+2 T→A	GGAGGCTGGATGATCCTTAAG	TAGCAGTGAGAATGCCGACC
112	*MRP2*	Gln1382Arg (CAG→CGG) in exon 29	AGAGATGGAGTAGCCAGTCAC	CAGCCACAAATGCATATTACC
113	*MTCO2*	Val142Met (GTA→ATA)	ATCCCTCCCTTACCATCA	GTCGTGTAGCGGTGAAA
114	*NOS2A*	+237A→G in exon 27 (3'-region)	CAAGTCTTATTTCCTCAACG	AATCTTTGTACACAAGGCAG
115	*NOS3*	–922A→G in 5'-region	AGTCTCTCAGCTTCCGTTT	CAGGTTCTCTCCTTCACC
116	*NOS3*	–786T→C in 5'-region	AGTCTCTCAGCTTCCGTTT	CAGGTTCTCTCCTTCACC
117	*p22 phox*	+24A→G in 3'-region	GAAGCCCAGCGAGGA	CTGAGAAGGAAGGCGATG
118	*PPARγ2*	Pro12Ala (CCA→GCA) in exon 1	TCAGTGTGAATTACAGCAAACCCC	CCCAATAGCCGTATCTGGAAGGAA
119	*PPARγ2*	His477His (CAC→CAT) in exon 6	AACCCCCTGTTGTGTTTCC	AGGTGTCAGATTTTCCCTCAG
120	*PPARγ2*	Pro115Gln (CCA→CAA) in exon 3	GCCTTTTAGGACTGTTTTCATGGG	TTCAATTGCCATGAGGGAGTTGGA
121	*RDH5*	Val200Val (GTC→GTG) in exon 4	CCTGGTCTGTGGTAACTTT	CCTGGGCACTCTTGTAA
122	*RDH5*	Gly238Trp (GGG→TGG) in exon 4	CCTGGTCTGTGGTAACTTT	CCTGGGCACTCTTGTAA
123	*RDH5*	Ser73Phe (TCC→TTC) in exon 2	GCCTTTGTCTTCATCACC	ACCCCACACCATATTCC
124	*SEP*	+14G→A in 3'-region	GTCAGTGACGTTTGCC	AAGCCAATTCAGTAGATTTC
125	*SOD1*	Gly38Arg (GGA→AGA) in exon 2	CTTCACTGTGAGGGGTAAAGGTAAATC	CTAACTAGGGTGAACAAGTATGGGTC
126	*SOD1*	Leu39Val (CTG→GTG) in exon 2	CTTCACTGTGAGGGGTAAAGGTAAATC	CTAACTAGGGTGAACAAGTATGGGTC
127	*SOD1*	Gly42Ser (GGC→AGC) in exon 2	CTTCACTGTGAGGGGTAAAGGTAAATC	CTAACTAGGGTGAACAAGTATGGGTC
128	*SOD1*	Gly42Asp (GGC→GAC) in exon 2	CTTCACTGTGAGGGGTAAAGGTAAATC	CTAACTAGGGTGAACAAGTATGGGTC
129	*SOD1*	His44Arg (CAT→CGT) in exon 2	CTTCACTGTGAGGGGTAAAGGTAAATC	CTAACTAGGGTGAACAAGTATGGGTC

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Appendix Table I—Cont'd

No.	*Gene*	*Polymorphism*	*Forward primer*	*Reverse primer*
130	*SOD1*	His47Arg (CAT→CGT) in exon 2	CTTCACTGTGAGGGGTAAAGGGTAAATC	CTAACTAGGGTGAACAAGTATGGGTC
131	*TNF*α	Position –1031 T→C in 5'-region	GGGGAGAACAAAAGGATAAGG	CCCCATACTCGACTTTCATAGC
132	*TNF*α	Position –863 C→A in 5'-region	CTCTGAGGAATGGGTTACAGGAGAC	GGCCCCAGTGTGTGGCCATATCTTC
133	*TNF*α	Position –857 C→T in 5'-region	CTCTGAGGAATGGGTTACAGGAGAC	GGCCCCAGTGTGTGGCCATATCTTC
134	*TNF*α	Position –376 G→A in 5'-region	CACAGGCCTCAGGACTCAACACAG	GGTCTTCTGGGCCACTGACTGATTT
135	*TNF*α	Position –308 G→A in 5'-region	TCTTTTTCCTGCATCCTGTCTGGAA	GTCTCGGTTTCTTCTCCATCGCGG
136	*TNF*α	Position –238 G→A in 5'-region	TCTTTTTCCTGCATCCTGTCTGGAA	GTCTCGGTTTCTTCTCCATCGCGG
137	*TNFR1*	Cys30Arg (TGT→CGT) in exon 2	TCTTCCCCAGGTGCTCC	GCCGATTCCCTGAAGTCTCT
138	*TNFR1*	Cys33Tyr (TGC→TAC) in exon 2	TCTTCCCCAGGTGCTCC	GCCGATTCCCTGAAGTCTCT
139	*TNFR1*	Thr50Met (ACG→ATG) in exon 3	CCCTCCCTCCTCTCCTA	CACCCACACACCACTCAAG
140	*TNFR1*	Cys52Phe (TGC→TTC) in exon 3	CCCTCCCTCCTCTCCTA	CACCCACACACCACTCAAG
141	*TNFR1*	Cys88Tyr, Arg (TGC→TAC, CGC) in exon 4	CACACACTTAGGGGTATGT	CTCAGGAGACTGCGCTCAC
142	*TNFR2*	Met196Arg (ATG→AGG) in exon 6	GGCACACATCGTCACTCT	AGCAGTGCTGGGTTCTG
143	*TPMT*	Tyr180Phe (TAT→TTT) in exon 8	AGCCTTTGCCTGTGTAGAG	ATATGCAGTATGCTTCCTATGAG
144	*TPMT*	Tyr240Cys (TAT→TGT) in exon 10	TGTTACTCTTTCTTGTTTCAGGTA	GCCATTTTTAGTAAAGATCTATCA
145	*TPMT*	Ala80Pro (GCA→CCA) in exon 5	CTTTGAAACCCTATGAACCTG	CCCAAATCAAAACAAACCTTA
146	*TPMT*	Glu98Stop (GAA→TAA) in exon 5	CTTTGAAACCCTATGAACCTG	CCCAAATCAAAACAAACCTTA

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Appendix Table I—Cont'd

No.	*Gene*	*Polymorphism*	*Forward primer*	*Reverse primer*
147	*TPMT*	Leu49Ser (TTA→TCA) in exon 4	TGTCTGAGCAAGTTACCTGTT	ATCCTGTTAAATCACCCAAAG
148	*TPMT*	(–1G→A) in intron 9	TAGAGACAGAGTTTCACCATCTTG	ATCCATTACATTTTCAGGCTTTAG
149	*TPMT*	His227Gln (CAT→CAG) in exon 10	ATCCCTGATGTCATTCTTCATAGT	ATCCATTACATTTTCAGGCTTTAG
150	*UCPI*	A→C at –113 in 5'-region	GCGGCCCAGCTATATAAGTC	ACCCCTTGTCTTACCCCTCTGC
151	*UCPI*	A→C at –180 in 5'-region	GCGGCCCAGCTATATAAGTC	ACCCCTTGTCTTACCCCTCTGC
152	*UCPI*	Ala64Thr in exon 2	AAAAGAAACGAAAATAGGAACAC	CCAATTTGTTATGAAAACCACTC
153	*UCPI*	Met229Leu in exon 5	TCTAGTGCAATACCTTTCTTATT	GCTTGTAAGCTAAATGTCG
154	*UCPI*	Val137Met in exon 3	GATAGCAGCACCTAGTTTAGGA	AACCCATTTTGAAGTTAGTTACC
155	*UGT1A1*	TA repeats (6/7)	AAAGTGAACTCCCTGCTACC	CAAAAACATTATGCCCGAGAC
156	*UGT1A1*	Gly71Arg in exon 1	AAAGTGAACTCCCTGCTACC	CAAAAACATTATGCCCGAGAC

Genes and their substances are as follows: *ADRB3*, β_3-adrenergic receptor; *ALB*, albumin; *APO*, apolipoprotein; *CASP9*, caspase 9; *CTLA4*, cytotoxic T-lymphocyte–associated molecule 4; *CYP*, cytochrome P450; *DT-diaphorase*, nicotinamide adenine dinucleotide phosphate, reduced:quinone acceptor oxidoreductase; *GLUT*, glucose transporter; *GPX*, glutathione peroxidase; *GST*, glutathione-S-transferase; *GYS*, glycogen synthase; *HGF*, hepatocyte growth factor; *IGF*, insulin-like growth factor; *IGFR*, insulin-like growth factor receptor; *IR*, insulin receptor; *IRS*, insulin receptor substrate; *LEP*, leptin; *LEPR*, leptin receptor; *LPL*, lipoprotein lipase; *MAOB*, monoamine oxidase B; *MRP2*, multidrug resistance–associated protein 2; *MTCO2*, cytochrome c oxidase 2; *NO*, nitric oxide; *PPARγ2*, peroxisome proliferator–activated receptor gamma 2; *RDH5*, retinol dehydrogenase 5; *SEP*, selenoprotein P; *SOD1*, superoxide dismutase 1; *TNFα*, tumor necrosis factor α; *TNFR*, tumor necrosis factor receptor; *TPMT*, thiopurine methyltransferase; *UCP1*, uncoupling protein 1; *UGT1A1*, uridine diphosphate–glucuronosyltransferase 1A1.