Genomics & Informatics Vol. 6(3) 99-109, September 2008

Protein Tyrosine Phosphatase N1 Gene Variants Associated with Type 2 Diabetes Mellitus and Its Related Phenotypes in the Korean Population

Kyung-Won Hong[1], Hyun-Seok Jin[1], Ji-Eun Lim[1], Ha-Jung Ryu[2], Younjhin Ahn[2], Jong-Young Lee[2], Bok Ghee Han[2], Hyoung Doo Shin[3,4], Nam Han Cho[5], Chol Shin[6], Jeong-Taek Woo[7], Hun Kuk Park[1] and Bermseok Oh[1]*

[1]Biomedical Education Center, Department of Biomedical Engineering, School of Medicine, KyungHee University, Seoul 130-701, Korea, [2]Center for Genome Science, National Institute of Health, Seoul 122-701, Korea, [3]SNP Genetics, Inc., Seoul 153-803, Korea, [4]Department of Life Science, Sogang University, Seoul 121-742, Korea, [5]Department of Preventive Medicine, Ajou University School of Medicine, Suwon 443-749, Korea, [6]Department of Internal Medicine, Korea University Ansan Hospital, Gyeonggi-do 425-707, Korea, [7]Department of Endocrinology and Metabolism, KyungHee University Hospital, School of Medicine, KyungHee University, Seoul 130-701, Korea

Abstract

Protein phosphorylation at tyrosine residues is a key regulatory event that modulates insulin signal transduction. We studied the *PTPN1* gene with regard to susceptibility to Korean type 2 diabetes mellitus (T2DM) and its related quantitative traits. A total of seven SNPs [g.36171G>A (rs941798), g.58166G>A (rs3787343), g.58208A>G (rs2909270), g.64840C>T (rs754118), g.69560C>G (rs6020612), g.69866G>A (rs718050), and g.69934T>G (rs3787343)] were selected based on frequency (>0.05), linkage disequilibrium (LD) status, and haplotype tagging status. We studied the seven SNPs in 483 unrelated patients with type 2 diabetes (age: 64±2.8 years, onset age: 56±8.1 years; 206 men, 277 women) and 1138 nondiabetic control subjects (age: 64±2.9; 516 men, 622 women). The SNP rs941798 had protective effects against T2DM with an odds ratio of 0.726 (C.I. 0.541~0.975) and p-value=0.034, but none of the remaining six SNPs was associated with T2DM. Also, rs941798 was associated with blood pressure, HDL cholesterol, insulin sensitivity. rs941798 also has been associated with T2DM in previous reports of Caucasian-American and Hispanic-American populations. This is the first report that shows an association between *PTPN1* and T2DM in the Korean as well as Asian population.

Keywords: *PTPN1*, insulin signaling, Korean, T2DM, rs941798

*Corresponding author: E-mail ohbs@khu.ac.kr
Tel +82-2-961-0290, Fax +82-2-961-5515
Accepted 2 September 2008

Introduction

Protein phosphorylation at tyrosine residues modulates intracellular signaling pathways, and this modulation is an essential determinant of insulin signal transduction (Goldstein *et al.*, 1998; Evans and Jallal, 1999). Protein tyrosine phosphatase 1B protein (PTP1B), encoded by the *PTPN1* gene, regulates the tyrosine phosphorylation of insulin receptor (Seely *et al.*, 1996) and insulin receptor substrate 1 (Goldstein *et al.*, 2000), which leads to downregulation of insulin signaling. *PTPN1* deficiency in mice results in increased insulin sensitivity (Elchebly *et al.*, 1999) and enables normalization of blood glucose levels (Klaman *et al.*, 2000). Moreover, it has been shown that inactivation with antisense oligonucleotides regulates the expression of genes that are involved in lipogenesis, such as SREBF1, suggesting that PTP1B may play a role in the enlargement of adipocyte energy storage (Rondinone *et al.*, 2002).

The human *PTPN1* gene maps to chromosome 20q13.13, a syntenic region of the distal arm of mouse chromosome 2 that harbors quantitative trait loci for body fat and body weight (Lembertas *et al.*, 1997). The PTPB1 gene consists of 10 exons, spanning 74 kb, and the first intron is longer than 50 kb. In humans, several linkage signals with type 2 diabetes mellitus (T2DM) (Bowden *et al.*, 1997), BMI (Hunt *et al.*, 2001), fat mass, and energetic intake (Collaku *et al.*, 2004; Dong *et al.*, 2003; Lembertas *et al.*, 1997) were reported at this locus in different populations, further supporting the candidacy of *PTPN1* involvement in T2DM and obesity. In Poland, a family-based linkage study of T2DM showed the highest logarithm of the odds score (Ji *et al.*, 1997; Klupa *et al.*, 2000). This locus also showed evidence of linkage with early onset T2DM (onset=45 years) in a subset of 55 French families (Zouali *et al.*, 1997).

Since the discovery of the *PTPN1* gene (Forsell *et al*., 2000), multiple studies have attempted to examine its role in susceptibility to T2DM. A missense variant in the coding region of *PTPN1* was found to be associated with T2DM and glucose tolerance in the Danish population (Echwald *et al*., 2002), and an insertion/deletion polymorphism in the 3'-untranslated region was associated with insulin resistance (Di Paola *et al*., 2002). In an extensive analysis of the *PTPN1* gene locus, Bento *et al*. (2004) observed associations between multiple SNPs and T2DM in two independent Caucasian-American case-control samples. The evidence for association was most consistent for SNPs in the region that spanned the 3'-end of intron 1 to the last, intron 8. All of the associated SNPs lay in a single haplotype block, and one common haplotype (frequency=36%) was found to be strongly associated with T2DM. The same group evaluated and confirmed previous findings of SNPs and haplotypes of *PTPN1* for association with quantitative glycemic traits in Hispanic-American subjects from the Insulin Resistance Atherosclerosis Study Family Study (IRASFS) (Palmer *et al*., 2004). However, a recent meta-analysis that included 7883 individuals from three large European case-control samples (from the US, Poland, and Scandinavia) did not replicate this association for any single SNP or haplotype (Florez *et al*., 2005). A conclusion of the association between *PTPN1* and T2DM in Asian and African populations is needed.

Despite the inconsistency of the association with T2DM, the evidence that *PTPN1* might be a significant contributor to genetic susceptibility to T2DM seems to be strong. Because no study of *PTPN1* in association with T2DM in the Asian population has been presented, we provide the first report of the susceptibility of the *PTPN1* gene in Korean T2DM and its related quantitative traits.

Methods

Subjects

The 24 DNA samples from Korean subjects that were used for the initial sequencing were randomly selected from unrelated local residents with no history of familial diseases. With 24 samples (48 chromosomes), it might be expected that more than 90% of SNPs have a frequency that is greater than 0.05 (Eberle and Kruglyak, 2000). We studied 483 unrelated patients with type 2 diabetes (age: 64±2.8 years, onset age: 56±8.1 years; 206 men, 277 women) and 1138 nondiabetic control subjects (age: 64±2.9; 516 men, 622 women). All subjects who enrolled in this study originated from the Ansung-Ansan prospective community cohort.

In this study, diabetic subjects were recruited according to American Diabetes Association criteria from 10,038 individuals in the Ansung-Ansan prospective community cohort. Nondiabetic subjects who had no history of diabetes, no first-degree relatives with diabetes, fasting plasma glucose levels less than 6.1 mmol/L, and hemoglobin A1c (HbA1c) levels less than 5.8% were recruited from the cohort. To be eligible, normal control subjects also had to be free of medications for diabetes, hypertension, and dyslipidemia. The study was approved by the institutional review board of the Korean National Institute of Health. Written informed consent was obtained from all subjects.

Measurement of clinical characteristics

Both T2DM and normal control subjects were aged 60 years and older. The clinical characteristics of the subjects are summarized in Table 1. All study subjects were examined in the morning after an overnight fast and 60 min and 120 min after an oral glucose tolerance test (OGTT). The parameters that were measured were height, weight, body mass index [BMI: weight (kg)/square of height (m^2)], waist and hip circumference, waist-to-hip ratio (WHR: waist/hip), and blood pressure. Blood samples were drawn for biochemical measurements: fasting plasma glucose (GLU0), OGTT 60 min and 120 min plasma glucose (GLU60 and GLU120, respectively), fasting plasma insulin (INS0), OGTT 60 min and 120 min plasma insulin (INS60 and INS120, respectively), HbA1c, total cholesterol (TCHL), triglycerides (TG), and high-density lipoprotein cholesterol (HDL). Using the measurements, low-density lipoprotein cholesterol [LDL: TCHL-TG-(HDL/5)], area under glucose curve (AUCGLU), area under insulin curve (AUCINS), homeostasis model assessment of insulin resistance [HOMA-IR: GLU0*INS0/(22.5*14.182)], quantitative insulin sensitivity check index [QUICKI: 1/(log(GLU0)+log(INS0)], and insulin sensitivity index (ISI) were calculated. The QUICKI was derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose levels. This index correlates well with glucose clamp studies (r=0.78) and is useful for measuring insulin sensitivity (IS), which is the inverse of insulin resistance (IR) (Katz *et al*., 2000).

Sequencing analysis of the human *PTPN1* gene

We sequenced all exons, including exon-intron boundaries and the promoter region (approximately 1.5 kb), to discover single nucleotide polymorphisms (SNPs) in 24 DNA samples from Koreans using the ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA,

USA). Sixteen primer sets for the amplification and sequencing analysis were designed based on GenBank sequences (Ref. Genome seq. NT_011362 released on February 19, 2004). Information regarding primers is available on our website (http://www.ngri.re.kr/SNP/). Sequence variants were verified by chromatograms.

Genotyping

Among the identified polymorphisms, seven SNPs (g.36171G>A, g.58166G>A, 58208A>G, g.64840C>T, g.69560C>G, g.69866G>A, and g.69934T>G) were selected based on frequency (>0.05), linkage disequilibrium (LD) status, and haplotype tagging status. In addition, one indel (g.1484G_ins) was selected based on previous reports (Bento *et al.*, 2004; Burdon *et al.*, 2006).

SNPs were genotyped using amplifying primers and probes designed for TaqMan (Livak, 1999). Primer Express (Applied Biosystems) was used to design both the PCR primers and the MGB TaqMan probes. One allelic probe was labeled with the FAM dye, and the other was labeled with the fluorescent VIC dye. PCRs were run in TaqMan Universal Master mix without UNG (Applied Biosystems) and with PCR primer concentrations of 900 nM and TaqMan MGB probe at a concentration of 200 nM. Reactions were performed in 384-well format in a total reaction volume of 5 μl using 20 ng of genomic DNA. The plates were then placed in a thermal cycler (PE 9700, Applied Biosystems) and heated at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min, with a final soak at 25°C. The TaqMan assay plates were transferred from the thermal cyclers to a real-time PCR system (Prism 7900HT, Applied Biosystems) that read the fluorescence intensity of each well of the plate. Fluorescence data files from each plate were analyzed using automated software (SDS ver. 2.1, Applied Biosystems).

Statistics

Differences in anthropometric and physiologic variables between nondiabetic and diabetic subjects were com-

Table 1. Clinical characteristics of study subjects

Variables	Abbreviation	Normal Control (n=1,138)			T2DM (n=483)			Test statistic*	p-value
		N	*Mean*	*SD*	*N*	*Mean*	*SD*		
Age (yrs)		1,138	64.237	2.873	483	64.621	2.779	−2.480	0.0130
Onset (yrs)					271	56.288	8.091		
Duration (mths)					271	101.181	94.071		
Sex (men/women)		516 (45.34)/622 (54.66)			206 (42.65)/277 (57.35)			0.995	0.3190
Body mass index (Kg/m^2)	BMI	1,138	23.660	3.143	483	25.128	3.154	−8.590	<.0001
Waist-to-hip ratio	WHR	1,137	0.913	0.066	482	0.932	0.067	−5.190	<.0001
Systolic blood pressure (mm Hg)	SBP	1,138	124.773	18.580	483	129.170	18.326	−4.380	<.0001
Diastolic blood pressure (mm Hg)	DBP	1,138	77.418	10.158	483	77.600	10.357	−0.330	0.7433
Triglyceride (mg/dL)	TG	1,138	147.679	72.202	483	199.887	140.273	−7.760	<.0001
Total cholesterol (mg/dL)	TCHL	1,138	185.431	33.947	483	194.884	42.264	−4.360	<.0001
High-density lipoprotein cholesterol (mg/dL)	HDL	1,138	45.208	10.193	483	42.678	9.857	4.610	<.0001
Low-density lipoprotein cholesterol (mg/dL)	LDL	1,125	111.080	31.568	452	115.586	38.382	−2.210	0.0270
Fasting plasma glucose (mg/dL)	GLU0	1,138	80.625	7.755	326	118.028	35.242	−19.030	<.0001
Plasma glucose after 60 min of oral glucose tolerence test (OGTT) (mg/dl)	GLU60	1,135	133.307	37.708	268	249.451	52.941	−33.940	<.0001
Plasma glucose after 120 min of OGTT (mg/dL)	GLU120	1,138	101.917	21.315	268	247.705	61.951	−38.000	<.0001
Area under glucose curve (mg/dL · hr)	AUCGLU	1,135	224.563	42.776	268	430.213	90.504	−36.250	<.0001
Fasting plasma insulin (μU/mL)	INS0	1,138	7.459	6.754	328	8.879	6.918	−3.340	0.0009
Plasma insulin after 60 min of OGTT (μU/mL)	INS60	1,135	33.320	31.813	267	26.245	28.176	3.340	0.0009
Plasma insulin after 120 min of OGTT (μU/mL)	INS120	1,138	23.436	24.317	267	33.386	40.686	−3.840	0.0001
Area under insulin curve (μU/mL · hr)	AUCINS	1,135	48.773	39.283	267	47.359	44.102	0.480	0.6308
Hemoglobin A1c (%)	HbA1c	1,138	5.486	0.241	483	7.355	1.534	−26.640	<.0001
Homeostasis model assessment of insulin resistance	HOMA-IR	1,138	1.493	1.364	326	2.578	1.959	−9.370	<.0001
Microalbuminuria (mg/d)	MALB_U	445	1.776	2.888	165	4.239	13.163	−2.380	0.0183

*Test statistics between normal and T2DM using student t-test for all variables except sex, whichwas compared by chi square test.

pared by student t-test for all variables, except sex, which was compared by the chi square test. Deviation of genotype frequency from the expected Hardy-Weinberg equilibrium was examined with the chi square test. To approximate a normal distribution, TG, INS 0, INS60, INS120, AUCINS, HOMA-IR, QUICKI, ISI, and MALB_U were log-transformed before analysis. We examined linkage disequilibrium (D') and generated a plot for the *PTPN1* gene using Haploview v3.2 (http://broad.mit.edu/haploview/) (Barrett *et al.*, 2005). Haplotypes were inferred using Haploview. Differences in genotype frequencies between T2DM patients and controls were compared using the chi square test, and the mode of inheritance was analyzed by a logistic regression procedure. Genotypes were given codes of 0, 1 and 2; 0, 1 and 1; 0, 0 and 1 in the additive, dominant, and recessive models, respectively. The associations between SNPs or haplotypes and T2DM-related subphenotypes were determined by linear regression analysis while controlling for age, sex, and BMI among normal control subjects. The SAS statistical software package (SAS Institute Inc. Cary, NC, USA) was used to perform general statistical analyses. Statistical significance was determined at a two-tailed value of $p < 0.05$.

Korean SNP database

Information on most of the SNPs that are described in this study is available in the Korean SNP database (http://www.ngri.re.kr/SNP/), which was constructed at the Center for Genome Sciences (Korean National Institute of Health).

Results

Most of the variables that are shown in Table 1 were significantly higher in T2DM patients than normal controls, but HDL cholesterol and INS60 [insulin level at 1 hour after oral glucose tolerance test (OGTT)] were lower in T2DM patients than in normal controls, and no significant differences were observed in diastolic blood pressure or AUCINS (area under the curve in insulin level during OGTT) between groups.

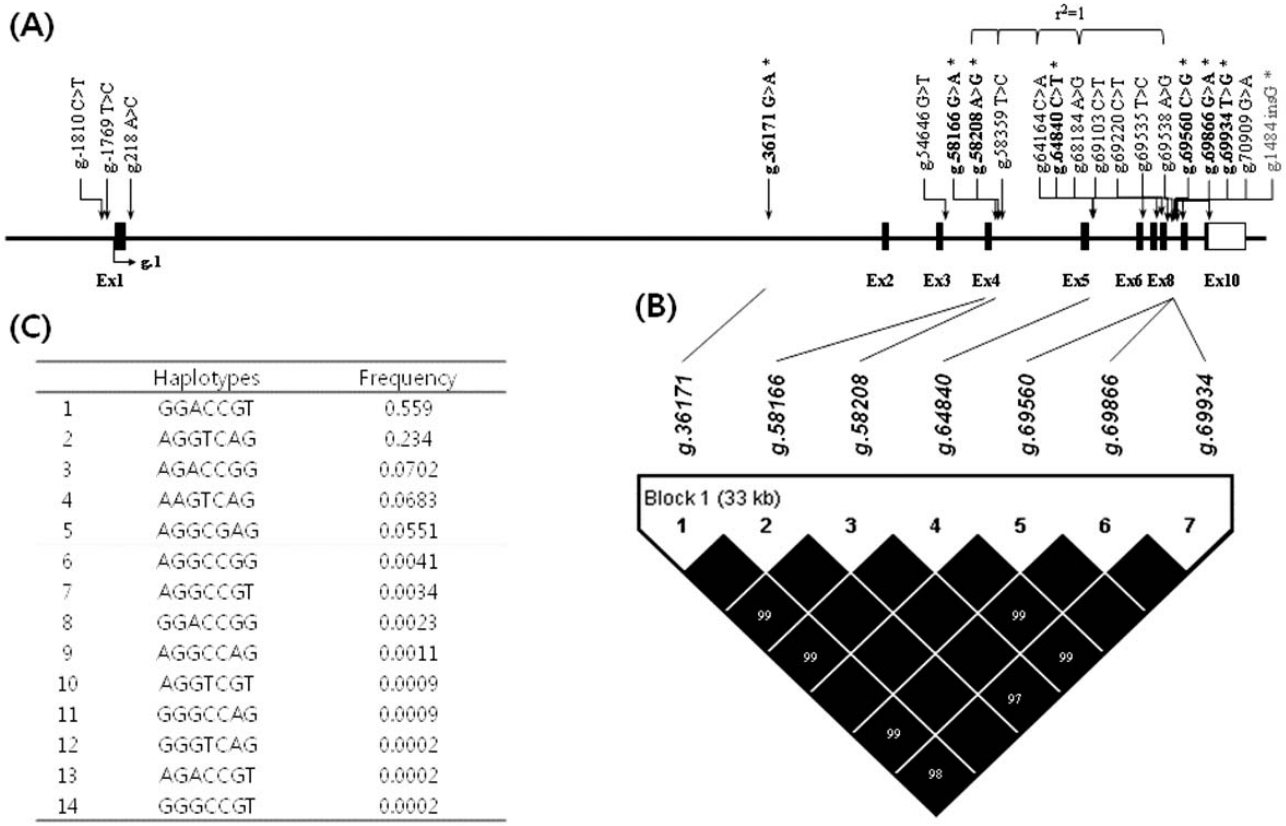
Through direct sequencing of all exons and their boundaries in the *PTPN1* gene, including up to -1500 bp of the 5' -flanking region, 19 SNPs were identified (Table 2). The genomic positions of the SNPs are illustrated in Fig. 1A. The SNPs that were selected for further study are in bold and with an asterisk (*); they had more than a 5% allele frequency and a tagging of the linkage disequilibrium (LD) block (data not shown).

None of the SNPs deviated from HWE (Hardy-

Table 2. Nineteen SNPs identified by direct sequencing in 24 unrelated subjects and one insertion/deletion (indel)

Type	Position	Position from TSS in gene	Position from TSS in coding region	Major Allele	Minor Allele	Minor allele Frequency	Matching with dbSNP	Position in exon	Amino acid position	Amino acid-wild type	Amino acid-mutant type
SNP	Promoter	−1810		C	T	0.022	novel				
SNP	Promoter	−1769		T	C	0.022	novel				
SNP	Intron 1	218		A	C	0.295	rs6067471				
SNP	Intron 1	36,171		G	A	0.354	rs941798				
SNP	Intron 3	54,646		G	T	0.021	novel				
SNP	Intron 4	58,166		G	A	0.063	rs3787343				
SNP	Intron 4	58,208		A	G	0.375	rs2904270				
SNP	Intron 4	58,359		T	C	0.375	rs3787345				
SNP	Intron 5	64,164		C	A	0.391	rs1885177				
SNP	Intron 5	64,840		C	T	0.302	rs754118				
SNP	Intron 6	68,184		A	G	0.409	rs968701				
SNP	Intron 7	69,103		G	A	0.313	rs2282147				
SNP	Exon 8	69,220	909	C	T	0.130	rs2282146	cds	303	Pro (CCC)	Pro (CCT)
SNP	Intron 8	69,535		T	C	0.391	rs718049				
SNP	Intron 8	69,538		A	G	0.391	rs718053				
SNP	Intron 8	69,560		C	G	0.065	rs6020612				
SNP	Intron 8	69,866		G	A	0.356	rs718050				
SNP	Intron 8	69,934		T	G	0.433	rs3787348				
SNP	E9	70,909	1,260	G	A	0.022	novel	cds	420	Thr (ACG)	Thr (ACA)
Insdel	Exon 10	72,363	1,484	G		0.000		3' UTR			

TSS: Translation start site, Underline: selected SNPs for further genotyping in Ansan-Ansung cohort participants.



(C)

	Haplotypes	Frequency
1	GGACCGT	0.559
2	AGGTCAG	0.234
3	AGACCGG	0.0702
4	AAGTCAG	0.0683
5	AGGCGAG	0.0551
6	AGGCCGG	0.0041
7	AGGCCGT	0.0034
8	GGACCGG	0.0023
9	AGGCCAG	0.0011
10	AGGTCGT	0.0009
11	GGGCCAG	0.0009
12	GGGTCAG	0.0002
13	AGACCGT	0.0002
14	GGGCCGT	0.0002

Fig. 1. (A) Map of PTPN1 (protein tyrosine phosphatase, non-receptor type 1) on chromosome 20q13.1-q13.2 (74 kb). Coding exons are marked by shaded blocks, and 5'- and 3'-untranslated regions are marked by white blocks. Asterisks (*) indicate SNPs that were genotyped in the larger population. The first nucleotide of the translational start site is denoted as 'nucleotide plus 1' (reference sequence of *PTPN1*: NT_011362). (B) Plot displaying linkage disequilibrium (LD) based on D' for the selected SNPs using the control population. All D' values were greater than 0.97. (C) Haplotype frequencies for seven SNPs selected from sequencing results and genotyped in normal control subjects.

Table 3. Logistic regression analysis for diabetic and the non-diabetic subjects with sex, age, and BMI adjustments

	Codominant		Dominant		Recessive	
	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
rs941798 (g.36171G>A)	0.929 (0.791~1.091)	0.369	1.059 (0.832~1.348)	0.642	0.726 (0.541~0.975)	0.034
rs3787343 (g.58166G>A)	0.801 (0.583~1.101)	0.171	0.810 (0.581~1.127)	0.211	0.365 (0.043~3.110)	0.356
rs2904270 (g.58208A>G)	0.991 (0.842~1.167)	0.916	1.071 (0.854~1.344)	0.553	0.838 (0.602~1.165)	0.293
rs754118 (g. 64840C>T)	1.000 (0.845~1.184)	0.997	1.026 (0.824~1.279)	0.817	0.929 (0.637~1.354)	0.701
rs6020612 (g.69560C>G)	0.771 (0.535~1.113)	0.165	0.761 (0.521~1.111)	0.157	0.846 (0.086~8.326)	0.886
rs718050 (g.69866G>A)	0.970 (0.823~1.143)	0.718	1.036 (0.826~1.298)	0.762	0.817 (0.582~1.147)	0.244
rs3787348 (g.69934T>G)	0.937 (0.798~1.101)	0.429	1.059 (0.832~1.348)	0.644	0.746 (0.555~1.002)	0.052

Underline indicates significant result with p-value<0.05.

Weinberg Equilibrium). An insertion/deletion variant (g.1484G_ins) was not polymorphic in our subjects. The selected seven SNPs belonged to an LD block with |D'| >0.98 (Fig. 1B). Five haplotypes with greater than a 5% frequency were predicted using the case-control samples (Fig. 1C). The results of the logistic regression analysis are described in Table 3. rs941798 had a protective effect against diabetes, with an odds ratio of 0.726 (C.I. 0.541~0.975) and p-value=0.034.

Table 4. Linear regression analysis between PTPN1 genotypes and insulin resistance index adjusted for age, sex, and BMI

	C (Major allele)/C	C/R (Risk allele)	R/R	Codominant	Dominant	Recessive
				p	p	p
rs941798						
GLU0	291 (80.82±8.03)	470 (80.48±7.86)	192 (80.51±7.76)	0.681	0.556	0.965
GLU60	290 (132.29±37.03)	468 (133.67±40)	192 (132.7±38)	0.671	0.593	0.895
GLU120	291 (102.15±21.23)	470 (100.59±22.1)	192 (102.07±21.38)	0.460	0.247	0.973
AUCGLU	290 (223.75±41.32)	468 (224.18±45.64)	192 (223.99±42.79)	0.871	0.891	0.899
lnINS0	291 (2.19±0.46)	470 (2.23±0.41)	192 (2.27±0.33)	0.105	0.138	0.253
lnINS60	290 (3.19±0.86)	468 (3.24±0.8)	192 (3.21±0.8)	0.802	0.545	0.798
lnINS120	291 (2.91±0.74)	470 (2.96±0.73)	192 (2.95±0.67)	0.910	0.603	0.690
lnAUCINS	290 (3.62±0.73)	468 (3.69±0.65)	192 (3.66±0.64)	0.694	0.343	0.691
HBA1C	291 (5.48±0.24)	470 (5.49±0.23)	192 (5.49±0.22)	0.635	0.587	0.835
lnHOMAIR	291 (1.45±0.23)	470 (1.47±0.2)	192 (1.48±0.17)	0.489	0.465	0.707
lnQUICKI	291 (1.22±0.03)	470 (1.22±0.02)	192 (1.22±0.01)	0.007	0.033	0.022
lnISI	290 (1.39±0.25)	468 (1.36±0.17)	192 (1.36±0.19)	0.114	0.029	0.788
rs3787343						
GLU0	812 (80.73±8.05)	132 (79.7±6.52)	6 (85.67±10.88)	0.645	0.385	0.093
GLU60	809 (133.8±38.73)	132 (128.96±38.1)	6 (139.17±35.92)	0.461	0.360	0.566
GLU120	812 (101.69±21.49)	132 (99.16±22.96)	6 (111.67±18.66)	0.224	0.136	0.383
AUCGLU	809 (224.99±43.67)	132 (218.39±43.53)	6 (237.83±41.65)	0.327	0.215	0.384
lnINS0	812 (2.22±0.41)	132 (2.23±0.4)	6 (2.31±0.09)	0.991	0.960	0.863
lnINS60	809 (3.21±0.82)	132 (3.24±0.83)	6 (3.42±0.95)	0.644	0.711	0.588
lnINS120	812 (2.94±0.73)	132 (2.93±0.69)	6 (2.64±0.72)	0.301	0.428	0.170
lnAUCINS	809 (3.66±0.68)	132 (3.68±0.68)	6 (3.73±0.77)	0.952	0.959	0.954
HBA1C	812 (5.49±0.24)	132 (5.5±0.23)	6 (5.45±0.19)	0.871	0.783	0.649
lnHOMAIR	812 (1.47±0.21)	132 (1.47±0.2)	6 (1.5±0.03)	0.764	0.730	0.911
lnQUICKI	812 (1.22±0.02)	132 (1.22±0.02)	6 (1.21±0)	0.610	0.682	0.549
lnISI	809 (1.37±0.2)	132 (1.38±0.21)	6 (1.36±0.27)	0.761	0.733	0.939
rs2904270						
GLU0	373 (80.95±8.14)	443 (80.44±7.72)	131 (80.31±7.84)	0.366	0.324	0.697
GLU60	372 (132.67±37.88)	441 (133.78±40.58)	131 (132.54±34.77)	0.715	0.629	0.971
GLU120	373 (102.42±21.05)	443 (100.09±22.54)	131 (102.23±21.48)	0.206	0.072	0.969
AUCGLU	372 (224.33±42.4)	441 (224.02±46.03)	131 (223.81±40.18)	0.946	0.918	0.990
lnINS0	373 (2.2±0.46)	443 (2.23±0.38)	131 (2.27±0.36)	0.265	0.365	0.358
lnINS60	372 (3.19±0.84)	441 (3.24±0.79)	131 (3.24±0.85)	0.585	0.537	0.837
lnINS120	373 (2.91±0.75)	443 (2.97±0.72)	131 (2.9±0.68)	0.656	0.800	0.215
lnAUCINS	372 (3.64±0.71)	441 (3.69±0.65)	131 (3.68±0.67)	0.642	0.483	0.942
HBA1C	373 (5.48±0.24)	443 (5.49±0.23)	131 (5.5±0.22)	0.606	0.573	0.823
lnHOMAIR	373 (1.46±0.23)	443 (1.46±0.18)	131 (1.48±0.19)	0.819	0.942	0.728
lnQUICKI	373 (1.22±0.03)	443 (1.22±0.02)	131 (1.22±0.01)	0.013	0.023	0.087
lnISI	372 (1.39±0.24)	441 (1.36±0.18)	131 (1.36±0.19)	0.202	0.101	0.838
rs754118						
GLU0	454 (80.73±7.96)	404 (80.7±7.98)	96 (79.54±7.05)	0.382	0.654	0.240
GLU60	453 (131.71±37.37)	402 (134.98±41.33)	96 (130.72±33.06)	0.461	0.264	0.813
GLU120	454 (101.81±21.42)	404 (100.87±22.14)	96 (101.54±21.2)	0.436	0.385	0.791
AUCGLU	453 (222.97±41.93)	402 (225.74±46.96)	96 (221.26±38.02)	0.704	0.467	0.708
lnINS0	454 (2.2±0.45)	404 (2.23±0.38)	96 (2.29±0.36)	0.114	0.193	0.192
lnINS60	453 (3.21±0.84)	402 (3.21±0.8)	96 (3.27±0.79)	0.645	0.856	0.479
lnINS120	454 (2.94±0.75)	404 (2.95±0.71)	96 (2.87±0.65)	0.364	0.711	0.169
lnAUCINS	453 (3.66±0.7)	402 (3.66±0.66)	96 (3.69±0.61)	0.858	0.960	0.756
HBA1C	454 (5.49±0.24)	404 (5.49±0.23)	96 (5.47±0.24)	0.540	0.822	0.330
lnHOMAIR	454 (1.46±0.22)	404 (1.47±0.18)	96 (1.48±0.19)	0.523	0.619	0.564
lnQUICKI	454 (1.22±0.03)	404 (1.22±0.02)	96 (1.22±0.01)	0.006	0.009	0.099
lnISI	453 (1.38±0.23)	402 (1.37±0.19)	96 (1.35±0.16)	0.213	0.282	0.344

Table 4. Continued

	C (Major allele)/C	C/R (Risk allele)	R/R	Codominant	Dominant	Recessive
				p	p	p
rs6020612						
GLU0	854 (80.55±7.87)	99 (80.98±7.78)	2 (74±11.31)	0.916	0.776	0.239
GLU60	851 (133.24±38.87)	99 (130.1±36.8)	2 (127±5.66)	0.421	0.424	0.842
GLU120	854 (101.43±21.68)	99 (100.64±22.74)	2 (99.5±24.75)	0.361	0.354	0.918
AUCGLU	851 (224.21±43.96)	99 (220.91±41.81)	2 (213.75±23.69)	0.359	0.369	0.759
lnINS0	854 (2.22±0.41)	99 (2.24±0.39)	2 (2.1±0.42)	0.775	0.816	0.682
lnINS60	851 (3.21±0.81)	99 (3.27±0.87)	2 (3.83±0.25)	0.511	0.605	0.285
lnINS120	854 (2.93±0.72)	99 (3.04±0.75)	2 (3.5±0.54)	0.215	0.268	0.254
lnAUCINS	851 (3.65±0.67)	99 (3.74±0.7)	2 (4.18±0.01)	0.326	0.398	0.259
HBA1C	854 (5.48±0.24)	99 (5.52±0.22)	2 (5.5±0.14)	0.195	0.184	0.952
lnHOMAIR	854 (1.46±0.21)	99 (1.47±0.18)	2 (1.39±0.2)	0.797	0.849	0.614
lnQUICKI	854 (1.22±0.02)	99 (1.22±0.02)	2 (1.22±0.02)	0.929	0.947	0.865
lnISI	851 (1.37±0.21)	99 (1.36±0.19)	2 (1.23±0.01)	0.548	0.636	0.325
rs718050						
GLU0	380 (80.93±8.13)	439 (80.39±7.72)	126 (80.25±7.68)	0.338	0.286	0.708
GLU60	379 (132.93±37.95)	437 (133.35±40.48)	126 (131.75±34.11)	0.922	0.853	0.942
GLU120	380 (102.19±21.18)	439 (100.54±22.22)	126 (102.01±21.19)	0.243	0.138	0.843
AUCGLU	379 (224.47±42.46)	437 (223.78±45.95)	126 (222.88±39.21)	0.777	0.768	0.889
lnINS0	380 (2.19±0.46)	439 (2.23±0.38)	126 (2.28±0.36)	0.148	0.229	0.247
lnINS60	379 (3.19±0.84)	437 (3.23±0.79)	126 (3.26±0.84)	0.523	0.549	0.680
lnINS120	380 (2.91±0.75)	439 (2.97±0.71)	126 (2.91±0.68)	0.792	0.667	0.251
lnAUCINS	379 (3.64±0.71)	437 (3.68±0.65)	126 (3.7±0.65)	0.583	0.515	0.874
HBA1C	380 (5.48±0.24)	439 (5.49±0.23)	126 (5.49±0.23)	0.817	0.729	0.969
lnHOMAIR	380 (1.46±0.23)	439 (1.47±0.18)	126 (1.48±0.19)	0.619	0.763	0.574
lnQUICKI	380 (1.22±0.03)	439 (1.22±0.02)	126 (1.22±0.01)	0.008	0.015	0.076
lnISI	379 (1.38±0.23)	437 (1.36±0.18)	126 (1.36±0.17)	0.160	0.118	0.578
rs3787348						
GLU0	295 (80.92±8.03)	467 (80.5±7.85)	191 (80.42±7.76)	0.518	0.437	0.809
GLU60	294 (132.74±37.29)	465 (133.62±39.89)	191 (132.23±37.97)	0.876	0.773	0.953
GLU120	295 (102.1±21.33)	467 (100.81±22.02)	191 (101.85±21.49)	0.441	0.292	0.889
AUCGLU	294 (224.23±41.51)	465 (224.25±45.56)	191 (223.37±42.69)	0.917	0.943	0.920
lnINS0	295 (2.18±0.45)	467 (2.23±0.41)	191 (2.27±0.33)	0.071	0.092	0.218
lnINS60	294 (3.18±0.86)	465 (3.24±0.8)	191 (3.22±0.79)	0.686	0.436	0.851
lnINS120	295 (2.9±0.74)	467 (2.96±0.73)	191 (2.95±0.67)	0.789	0.481	0.732
lnAUCINS	294 (3.62±0.72)	465 (3.69±0.66)	191 (3.66±0.63)	0.598	0.281	0.752
HBA1C	295 (5.48±0.24)	467 (5.49±0.23)	191 (5.49±0.22)	0.626	0.568	0.842
lnHOMAIR	295 (1.45±0.22)	467 (1.47±0.2)	191 (1.48±0.17)	0.415	0.382	0.670
lnQUICKI	295 (1.22±0.03)	467 (1.22±0.02)	191 (1.22±0.01)	0.006	0.026	0.021
lnISI	294 (1.39±0.25)	465 (1.36±0.18)	191 (1.36±0.18)	0.088	0.025	0.672

Underline indicates significant result with p-value < 0.05.

The insulin resistance index had no significant association, except for log-transformed QUICKI and ISI (Table 4). The SNPs rs941798, rs2904270, rs9417114, rs718050, and rs3787348 showed significant association with QUICKI, but the differences were not distinguishable. The SNPs rs941798 and rs3787348 revealed a significant association with ISI, in which the individuals with minor alleles showed a decreased ISI.

Other quantitative traits that were related to T2DM were analyzed. Although there was no significant result between *PTPN1* and the obesity indices (BMI and WHR) (data not shown), significant associations with blood pressure were observed for six SNPs (rs941798, rs2904270, rs754118, rs6020612, rs718050, and rs3787348) (Table 5). Individuals that had risk alleles of the six SNPs had significantly increased systolic and diastolic blood pressures. In addition, SNPs rs941798, rs2904270, rs754118, rs718050, and rs3787348 were associated with decreased HDL cholesterol levels in the codominant or dominant model (Table 6). SNP rs6020612 re-

Table 5. Linear regression analysis between PTPN1 genotypes and blood pressure adjusted for age, sex, and BMI in non-diabetic subjects

	C/C	C/R	R/R	Codominant	Dominant	Recessive
				p	p	p
rs941798						
SBP	291 (120.49±16)	470 (122.34±17.72)	192 (124.67±17.57)	0.012	0.049	0.032
DBP	291 (75.15±9.45)	470 (76.09±9.65)	192 (78.23±9.87)	0.001	0.032	0.002
rs3787343						
SBP	812 (122.17±17.05)	132 (122.89±18.59)	6 (114.78±11.5)	0.901	0.888	0.225
DBP	812 (76.21±9.52)	132 (76.57±10.77)	6 (71.67±6.73)	0.922	0.867	0.227
rs2904270						
SBP	373 (121.03±16.12)	443 (121.95±17.64)	131 (125.68±18.27)	0.025	0.165	0.013
DBP	373 (75.44±9.4)	443 (76.1±9.5)	131 (78.42±10.77)	0.008	0.090	0.005
rs754118						
SBP	454 (121.8±16.88)	404 (121.93±17.2)	96 (125.5±18.75)	0.135	0.487	**0.033**
DBP	454 (75.69±9.48)	404 (76.45±9.73)	96 (77.84±10.26)	0.042	0.116	0.065
rs6020612						
SBP	854 (121.72±17.1)	99 (126.27±17.6)	2 (139±26.87)	0.017	0.025	0.141
DBP	854 (76.01±9.53)	99 (77.78±10.71)	2 (86.67±9.43)	0.076	0.110	0.117
rs718050						
SBP	380 (121.11±16.56)	439 (121.92±17.39)	126 (126.47±18.34)	0.014	0.168	0.003
DBP	380 (75.54±9.47)	439 (76.08±9.47)	126 (78.8±10.91)	0.006	0.110	0.002
rs3787348						
SBP	295 (120.56±16.17)	467 (122.19±17.66)	191 (124.76±17.49)	0.012	0.066	0.021
DBP	295 (75.27±9.42)	467 (76.02±9.71)	191 (78.16±9.81)	0.003	0.059	0.002

Underline indicates significant result with p-value < 0.05.

vealed increased triglycerides in the recessive model, but there were only 2 subjects that were homozygous for the minor allele.

Discussion

In this study, we investigated the effect of seven *PTPN1* SNPs on susceptibility to T2DM and its related quantitative traits in a Korean Ansung-Ansan prospective community cohort. Our T2DM association results are similar to those that were found for the Caucasian-American population (Bento *et al*., 2004) and Hispanic-American population (Palmer *et al*., 2004) but differ from a European study (Florez *et al*., 2005), which did not find any significant associations even though they obtained the same haplotype block.

A total of five SNPs consisting of haplotype 2 (Fig. 1C) showed a significant association with QUICKI. Two (rs941798 and rs3787348) of the five SNPs also were associated with ISI. The results imply that the association between *PTPN1* and T2DM may be caused by insulin resistance. Although the association with QUICKI was significant, the differences between genotypes were not clear. However, the ISI was decreased in individuals who had at least one minor allele. This result is well supported by a previous report (Palmer *et al*., 2004).

The association between the *PTPN1* gene and blood pressure was greatly in concordance with previous two reports (Cheyssac *et al*., 2006; Spencer-Jones *et al*., 2005). Both reports suggested that *PTPN1* gene variants increase blood pressure. Moreover, our results showed a more significant increase in systolic blood pressure in homozygotes of the SNP rs718050 minor allele. Although *PTPN1* is an important regulator of the insulin signaling pathway, these results imply that SNP rs718050 may be a useful marker to predict hypertension.

We also identified the increased tendency of TG in homozygotes of rs6020612; the minor allele homozygotes were observed in only two individuals. Although this association was reported in other reports (Cheyssac *et al*., 2006; Spencer-Jones *et al*., 2005), the associated SNP had a higher frequency of minor allele homozygotes. Therefore, the association between *PTPN1* and TG should be confirmed using other SNPs that have been previously reported.

PTP1B also inhibits leptin signaling through the dephosphorylation of JAK2 and STAT3 (Zabolotny *et al*., 2002; Cheng *et al*., 2002). Moreover, it was shown that inactivation with antisense oligonucleotides regulates the

Table 6. Linear regression analysis between PTPN1 genotypes and lipidemic index adjusted for age, sex, and BMI in non-diabetic subjects

	C/C	C/R	R/R	Codominant	Dominant	Recessive
				p	p	p
rs941798						
lnTG	291 (4.86±0.41)	470 (4.92±0.41)	192 (4.92±0.44)	0.187	0.080	0.757
TCHL	291 (185±33.88)	470 (183.84±33.45)	192 (183.52±32.69)	0.293	0.348	0.443
HDL	291 (47.12±11.04)	470 (45.09±9.57)	192 (44.16±9.51)	0.002	0.002	0.064
LDL	290 (110.44±32.27)	465 (109.73±31.03)	189 (110.41±29.44)	0.537	0.515	0.737
rs3787343						
lnTG	812 (4.9±0.42)	132 (4.89±0.4)	6 (4.78±0.55)	0.621	0.714	0.476
TCHL	812 (183.55±33.45)	132 (188.62±32.93)	6 (172.51±40.73)	0.474	0.303	0.233
HDL	812 (45.55±10.23)	132 (45.51±9.63)	6 (48.14±3.09)	0.728	0.837	0.464
LDL	803 (109.45±31.01)	132 (114.73±30.67)	6 (98.09±49.65)	0.440	0.260	0.179
rs2904270						
lnTG	373 (4.87±0.39)	443 (4.93±0.42)	131 (4.92±0.45)	0.107	0.052	0.662
TCHL	373 (183.53±33.69)	443 (184.61±33.07)	131 (185.5±34.47)	0.937	0.898	0.979
HDL	373 (46.41±10.56)	443 (45.25±9.64)	131 (44.14±10.09)	**0.038**	0.066	0.136
LDL	372 (109.72±32.12)	438 (110.17±30.45)	129 (111.81±30.71)	0.995	0.925	0.885
rs754118						
lnTG	454 (4.88±0.4)	404 (4.91±0.42)	96 (4.95±0.43)	0.139	0.208	0.247
TCHL	454 (184.21±33.04)	404 (184.05±33.68)	96 (184.65±34.5)	0.763	0.770	0.860
HDL	454 (46.16±10.51)	404 (45.3±9.62)	96 (43.8±9.91)	**0.049**	0.113	0.090
LDL	451 (110.06±31.21)	400 (110±31.34)	94 (110.85±29.86)	0.793	0.797	0.883
rs6020612						
lnTG	854 (4.9±0.41)	99 (4.91±0.46)	2 (5.67±0.07)	0.644	0.939	0.007
TCHL	854 (184.06±33.54)	99 (185.93±32.65)	2 (184.35±2.63)	0.979	0.980	0.986
HDL	854 (45.65±10.04)	99 (44.76±10.33)	2 (32.68±1.71)	0.321	0.453	0.069
LDL	848 (109.93±31.26)	96 (112.73±29.65)	2 (93.94±4.92)	0.889	0.797	0.454
rs718050						
lnTG	380 (4.87±0.4)	439 (4.91±0.42)	126 (4.95±0.45)	0.082	0.130	0.196
TCHL	380 (183.65±33.82)	439 (184.49±32.95)	126 (184.85±34.39)	0.847	0.941	0.779
HDL	380 (46.34±10.53)	439 (45.38±9.67)	126 (43.43±9.96)	0.016	0.073	0.027
LDL	379 (109.62±32.25)	434 (110.3±30.23)	123 (111.46±30.65)	0.931	0.938	0.950
rs3787348						
lnTG	295 (4.87±0.41)	467 (4.91±0.41)	191 (4.92±0.44)	0.254	0.207	0.579
TCHL	295 (185.11±34.03)	467 (184.09±33.59)	191 (183.1±32.3)	0.241	0.345	0.330
HDL	295 (47.07±11.08)	467 (45.18±9.54)	191 (44.07±9.55)	0.002	0.003	0.044
LDL	294 (110.29±32.51)	462 (110.19±31.08)	188 (109.87±29.1)	0.503	0.632	0.531

Underline indicates significant result with p-value < 0.05.

expression of genes that are involved in lipogenesis, such as SREBF1, suggesting that PTP1B may play a role in the enlargement of adipocyte energy storage (Rondinone *et al.*, 2002). Two SNPs (rs941798 and rs3787348) effected decreased HDL cholesterol levels in minor allele homozygotes. This result also has been replicated in another report (Cheyssac *et al.*, 2006), but the previous report showed a marginal association, while our results revealed lower p-values (rs941794 p=0.002, and rs3787348 p=0.003). Both SNPs showed a significant association with blood pressure, implying that the increased blood pressure might result in low HDL cholesterol levels and be related to lipid metabolism, which is another molecular pathway of *PTPN1* function (Santaniemi *et al.*, 2004).

This is the first report that shows the association between *PTPN1* and T2DM in the Korean as well as Asian population. We hope this study will increase our knowledge about T2DM pathophysiology.

Acknowledgments

This study was supported by an intramural grant from the National Institute of Health, Korea and an intramural grant from Kyung Hee University.

References

Benchabane, H., and Wrana, J.L. (2003). GATA- and Smad1-dependent enhancers in the Smad7 gene differentially interpret bone morphogenetic protein concentrations. *Mol. Cell. Biol.* 23, 6646-6661.

Bento, J.L., Palmer, N.D., Mychaleckyj, J.C., Lange, L.A., Langefeld, C.D., Rich, S.S., Freedman, B.I., and Bowden, D.W. (2004). Association of protein tyrosine phosphatase 1B gene polymorphisms with type 2 diabetes. *Diabetes* 53, 3007-3012.

Bowden, D.W., Sale, M., Howard, T.D., Qadri, A., Spray, B.J., Rothschild, C.B., Akots, G., Rich, S.S., and Freedman, B.I. (1997). Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. *Diabetes* 46, 882-886.

Burdon, K.P., Bento, J.L., Langefeld, C.D., Campbell, J.K., Carr, J.J., Wagenknecht, L.M., Herrington, D.M., Freedman, B.I., Rich, S.S., and Bowden, D.W. (2006). Association of protein tyrosine phosphatase-N1 polymorphisms with coronary calcified plaque in the Diabetes Heart Study. *Diabetes* 55, 651-658.

Cheng, A., Uetani, N., Simoncic, P.D., Chaubey, V.P., Lee-Loy, A., McGlade, C.J., Kennedy, B.P., and Tremblay, M.L. (2002). Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev. Cell* 2, 497-503.

Cheyssac, C., Lecoeur, C., Dechaume, A., Bibi, A., Charpentier, G., Balkau, B., Marre, M., Froguel, P., Gibson, F., and Vaxillaire, M. (2006). Analysis of common PTPN1 gene variants in type 2 diabetes, obesity and associated phenotypes in the French population. *BMC Med. Genet.* 7, 44.

Collaku, A., Rankinen, T., Rice, T., Leon, A.S., Rao, D.C., Skinner, J.S., Wilmore, J.H., and Bouchard, C. (2004). A genome-wide linkage scan for dietary energy and nutrient intakes: the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study. *Am. J. Clin. Nutr.* 79, 881-886.

Di Paola, R., Frittitta, L., Miscio, G., Bozzali, M., Baratta, R., Centra, M., Spampinato, D., Santagati, M.G., Ercolino, T., Cisternino, C., Soccio, T., Mastroianno, S., Tassi, V., Almgren, P., Pizzuti, A., Vigneri, R., and Trischitta, V. (2002). A variation in 3' UTR of hPTP1B increases specific gene expression and associates with insulin resistance. *Am. J. Hum. Genet.* 70, 806-812.

Dong, C., Wang, S., Li, W.D., Li, D., Zhao, H., and Price, R.A. (2003). Interacting genetic loci on chromosomes 20 and 10 influence extreme human obesity. *Am. J. Hum. Genet.* 72, 115-124.

Eberle, M. A., and Kruglyak, L. (2000). An analysis of strategies for discovery of single-nucleotide polymorphisms. *Genet. Epidemiol.* 19, Suppl. 1, S29-35.

Echwald, S.M., Bach, H., Vestergaard, H., Richelsen, B., Kristensen, K., Drivsholm, T., Borch-Johnsen, K., Hansen, T., and Pedersen, O. (2002). A P387L variant in protein tyrosine phosphatase-1B (PTP-1B) is associated with type 2 diabetes and impaired serine phosphorylation of PTP-1B in vitro. *Diabetes* 51, 1-6.

Elchebly, M., Payette, P., Michaliszyn, E., Cromlish, W., Collins, S., Loy, A.L., Normandin, D., Cheng, A., Himms-Hagen, J., Chan, C.C., Ramachandran, C., Gresser, M.J., Tremblay, M.L., and Kennedy, B.P. (1999). Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 283, 1544-1548.

Evans, J.L., and Jallal, B. (1999). Protein tyrosine phosphatases: their role in insulin action and potential as drug targets. *Expert Opin. Investig. Drugs.* 8, 139-160.

Florez, J.C., Agapakis, C.M., Burtt, N.P., Sun, M., Almgren, P., Rastam, L., Tuomi, T., Gaudet, D., Hudson, T.J., Daly, M.J., Ardlie, K.G., Hirschhorn, J.N., Groop, L., and Altshuler, D. (2005). Association testing of the protein tyrosine phosphatase 1B gene (PTPN1) with type 2 diabetes in 7,883 people. *Diabetes* 54, 1884-1891.

Forsell, P.A., Boie, Y., Montalibet, J., Collins, S., and Kennedy, B.P. (2000). Genomic characterization of the human and mouse protein tyrosine phosphatase-1B genes. *Gene* 260, 145-153.

Goldstein, B.J., Bittner-Kowalczyk, A., White, M.F., and Harbeck, M. (2000). Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. *J. Biol. Chem.* 275, 4283-4289.

Goldstein, B.J., Li, P.M., Ding, W., Ahmad, F., and Zhang, W.R. (1998). Regulation of insulin action by protein tyrosine phosphatases. *Vitam. Horm.* 54, 67-96.

Hunt, S.C., Abkevich, V., Hensel, C.H., Gutin, A., Neff, C.D., Russell, D.L., Tran, T., Hong, X., Jammulapati, S., Riley, R., Weaver-Feldhaus, J., Macalma, T., Richards, M.M., Gress, R., Francis, M., Thomas, A., Frech, G.C., Adams, T.D., Shattuck, D., and Stone, S. (2001). Linkage of body mass index to chromosome 20 in Utah pedigrees. *Hum. Genet.* 109, 279-285.

Ji, L., Malecki, M., Warram, J.H., Yang, Y., Rich, S.S., and Krolewski, A.S. (1997). New susceptibility locus for NIDDM is localized to human chromosome 20q. *Diabetes* 46, 876-881.

Katz, A., Nambi, S.S., Mather, K., Baron, A.D., Follmann, D.A., Sullivan, G., and Quon M.J. (2000). Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J. Clin. Endocrinol. Metab.* 85, 2402-2410.

Klaman, L.D., Boss, O., Peroni, O.D., Kim, J.K., Martino, J.L., Zabolotny, J.M., Moghal, N., Lubkin, M., Kim, Y.B., Sharpe, A.H., Stricker-Krongrad, A., Shulman, G.I., Neel, B.G., and Kahn, B.B. (2000). Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol. Cell Biol.* 20, 5479-5489.

Klupa, T., Malecki, M.T., Pezzolesi, M., Ji, L., Curtis, S., Langefeld, C.D., Rich, S.S., Warram, J.H., and Krolewski, A.S. (2000). Further evidence for a susceptibility locus for type 2 diabetes on chromosome 20q13.1-q13.2. *Diabetes* 49, 2212-2216.

Kwan, M., Powell, D.R., Nachman, T.Y., and Brown, M.A.

(2005). An intron GATA-binding site regulates chromatin accessibility and is essential for IL-4 gene expression in mast cells. *Eur. J. Immunol.* 35, 1267-1274.

Lembertas, A.V., Perusse, L., Chagnon, Y.C., Fisler, J.S., Warden, C.H., Purcell-Huynh, D.A., Dionne, F.T., Gagnon, J., Nadeau, A., Lusis, A.J., and Bouchard, C. (1997). Identification of an obesity quantitative trait locus on mouse chromosome 2 and evidence of linkage to body fat and insulin on the human homologous region 20q. *J. Clin. Invest.* 100, 1240-1247.

Livak, K.J. (1999). Allelic discrimination using Xuorogenic probes and the 5_nuclease assay. *Genet. Anal.* 14, 143-149

Palmer, N.D., Bento, J.L., Mychaleckyj, J.C., Langefeld, C.D., Campbell, J.K., Norris, J.M., Haffner, S.M., Bergman, R.N., and Bowden, D.W. (2004). Association of protein tyrosine phosphatase 1B gene polymorphisms with measures of glucose homeostasis in Hispanic Americans: the insulin resistance atherosclerosis study (IRAS) family study. *Diabetes* 53, 3013-3019.

Rondinone, C.M., Trevillyan, J.M., Clampit, J., Gum, R. J., Berg, C., Kroeger, P., Frost, L., Zinker, B.A., Reilly, R., Ulrich, R., Butler, M., Monia, B.P., Jirousek, M.R., and Waring, J.F. (2002). Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. *Diabetes* 51, 2405-2411.

Santaniemi, M., Ukkola, O., and Kesaniemi, Y.A. (2004). Tyrosine phosphatase 1B and leptin receptor genes and their interaction in type 2 diabetes. *J. Intern. Med.* 256, 48-55.

Seely, B.L., Staubs, P.A., Reichart, D.R., Berhanu, P., Milarski, K.L., Saltiel, A.R., Kusari, J., and Olefsky, J.M. (1996). Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. *Diabetes* 45, 1379-1385.

Spencer-Jones, N.J., Wang, X., Snieder, H., Spector, T.D., Carter, N.D., and O'Dell, S.D. (2005). Protein tyrosine phosphatase-1B gene PTPN1: selection of tagging single nucleotide polymorphisms and association with body fat, insulin sensitivity, and the metabolic syndrome in a normal female population. *Diabetes* 54, 3296-3304.

Zabolotny, J.M., Bence-Hanulec, K.K., Stricker-Krongrad, A., Haj, F., Wang, Y., Minokoshi, Y., Kim, Y.B., Elmquist, J.K., Tartaglia, L.A., Kahn, B.B., and Neel, B.G. (2002). PTP1B regulates leptin signal transduction in vivo. *Dev. Cell* 2, 489-495.

Zouali, H., Hani, E.H., Philippi, A., Vionnet, N., Beckmann, J.S., Demenais, F., and Froguel, P. (1997). A susceptibility locus for early-onset non-insulin dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. *Hum. Mol. Genet.* 6, 1401-1408.