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## Brief Genetics Report

# Association of Protein Tyrosine Phosphatase 1B Gene Polymorphisms With Measures of Glucose Homeostasis in Hispanic Americans

## The Insulin Resistance Atherosclerosis Study (IRAS) Family Study

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**Protein tyrosine phosphatase (PTP)-1B, encoded by the PTPN1 gene, catalyzes the dephosphorylation of proteins at tyrosyl residues. PTP-1B has been implicated in negatively regulating insulin signaling by dephosphorylating the phosphotyrosine residues of the insulin receptor. The genetic contribution of PTPN1 to measures of glucose homeostasis has been assessed in 811 Hispanic subjects from the Insulin Resistance Atherosclerosis Study Family Study (IRASFS). Thirty-five single nucleotide polymorphisms (SNPs) spanning 161 kb and containing the PTPN1 gene were genotyped and tested for association. All 20 SNPs with minor allele frequencies >0.1 in a single haplotype block covering the PTPN1 genomic sequence show significant association with the insulin sensitivity index ( $S_i$ ) ( $P = 0.044-0.003$ ) and fasting glucose ( $P = 0.029$  to  $<0.001$ ). In contrast, there is no evidence for association of PTPN1 polymorphisms with acute insulin response (a measure of  $\beta$ -cell function). Haplotype analysis of eight SNP haplotypes that have independently been shown to be associated with type 2 diabetes risk and protection in Caucasian type 2 diabetic subjects are associated with**

**lower ( $P = 0.007$ ) and higher ( $P = 0.0002$ )  $S_i$  and higher ( $P = 0.00007$ ) and lower ( $P = 0.001$ ) fasting glucose, respectively, in the IRASFS. This comprehensive genetic analysis of PTPN1 reveals significant association with metabolic traits consistent with the proposed in vivo role for the PTP-1B protein. *Diabetes* 53: 3013-3019, 2004**

**P**rotein phosphorylation at tyrosine is a key regulatory event that modulates intracellular signaling pathways involved in signal transduction. Protein tyrosine phosphatase (PTP)-1B is a ubiquitously expressed protein (1) that catalyzes the dephosphorylation of proteins at tyrosyl residues. PTP-1B has been implicated (2-4) in negatively regulating insulin signaling by dephosphorylating the phosphotyrosine residues of the insulin receptor kinase activation segment of the insulin receptor. In mouse models, disruption of the PTPN1 gene resulted in increased insulin sensitivity and resistance to diet-induced obesity (5,6). Further evidence for the role of PTP-1B in insulin sensitivity is seen in knockout mice, in which there was increased phosphorylation of the insulin receptor in liver and muscle tissue (5,6). These observations suggest that PTP-1B plays a role in modulating signal transduction, and defects in PTP-1B expression could lead to insulin resistance.

The 10 exons of PTPN1 span >74 kb of chromosome 20q13.13, with the first intron containing >50 kb of the sequence. Several investigators (7-9) have searched PTPN1 for DNA sequence variants, e.g., single nucleotide polymorphisms (SNPs). Variation within the coding region of PTPN1 is relatively uncommon. Echwald et al. (7) identified a P387L variant that was found in 2.6% of type 2 diabetic individuals and 1% of healthy control subjects, which showed evidence of impaired in vitro serine phosphorylation of the PTP-1B peptide. Mok et al. (8) identified a 981C→T polymorphism (5% minor allele frequency) that corresponded to a silent mutation in the PTP-1B protein

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AIR, acute insulin response; GEE1, generalized estimating equations; IRASFS, Insulin Resistance Atherosclerosis Study Family Study; LD, linkage disequilibrium; PTP, protein tyrosine phosphatase; SNP, single nucleotide polymorphism; UTR, untranslated region.

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and showed evidence of association with impaired glucose tolerance or type 2 diabetes in the Oji-Cree Indian tribe. Most recently, Di Paola et al. (9) reported evidence of an association between a 3' untranslated region (UTR) SNP located at position 1484 of the cDNA and several features associated with insulin resistance, including the homeostasis model assessment of insulin resistance index, serum triglycerides, and total-to-HDL cholesterol ratio. These investigators (9) presented further evidence that this SNP, denoted 1484insG, is functional and leads to overexpression of PTP-1B mRNA in skeletal muscle due to increased stability in in vitro experiments.

Quantitative trait analysis offers a powerful approach to identify variants that contribute to specific components of biochemical pathways. We have systematically evaluated the association of the PTPN1 gene and quantitative measures of glucose homeostasis in Hispanic subjects extensively phenotyped for measures of glucose homeostasis in the Insulin Resistance Atherosclerosis Study Family Study (IRASFS). We hypothesized that variants within PTPN1 will be associated with quantitative traits that are dependent on or ultimately reflect the actions of the insulin receptor, consistent with the proposed role of PTP-1B in vivo.

We identified 35 SNPs that comprehensively covered the genomic region containing PTPN1 (Fig. 1). The majority of the SNPs had a minor allele frequency  $>0.20$  and were found in noncoding regions. The average SNP density was 1 SNP/4.7 kb, with the largest gap between SNPs being 19.4 kb and the smallest 4 bp. Genotype frequencies were consistent with Hardy-Weinberg proportions.

Pairwise  $D'$  was calculated for the PTPN1 SNPs. When using  $D' > 0.80$  as criteria for evidence of significant linkage disequilibrium (LD), SNPs from rs4811077 to rs1060402, which spans 94 kb of sequence and encompasses the entire PTPN1 gene (data supplement [available at <http://diabetes.diabetesjournals.org>]), fall into a single LD haplotype block. A second, less well-defined block is comprised of SNPs 5' to PTPN1 from rs1967439 to rs4811075, containing 22 kb of sequence.

Results of the association analysis are shown in Tables 1 (insulin sensitivity index [ $S_i$ ]), 2 (fasting glucose), and 3 (acute insulin response [AIR]). Each table lists the SNPs in order of 5' to 3' across PTPN1, the SNP alleles (e.g., G/C), the minor allele frequency, the mean trait values and SDs for each genotype, and the  $P$  value for association between trait and SNP from the GEE1 (generalized estimating equation) analysis. Results are reported for SNPs with minor allele frequencies  $>0.1$  and the 1484insG SNP. As shown in Table 1, all 20 SNPs from rs3787334 (just distal to exon 1) through 1484insG (in the 3' UTR) are significantly associated with  $S_i$  ( $P = 0.044-0.003$ ). A similar pattern of association is seen with fasting glucose (Table 2), in which all 22 SNPs between rs718630 (proximal to PTPN1 exon 1) and rs1060402 (distal to exon 10) show significant evidence of association ( $P = 0.029$  to  $<0.0001$ ). In contrast, there is little or no evidence of association with AIR, a measure of  $\beta$ -cell function (Table 3), with only 2 (1484insG and rs4811077) of 29 SNPs showing evidence of association.

**Haplotype analysis.** Tables 4 and 5 show the results of eight SNP haplotype analyses (rs941798, rs3787345, rs754118, rs2282147, rs718049, rs718050, rs3787348, and 1484insG) with  $S_i$  (Table 4) and fasting glucose (Table 5),

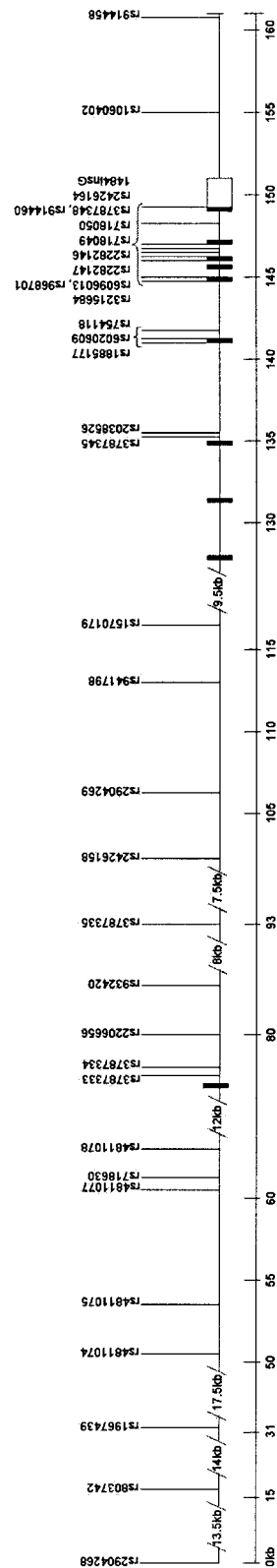


FIG. 1. Genomic map of PTPN1 gene with locations of the genotyped SNPs. The shaded regions are exons, numbered 1–10. The ruler along the bottom represents the relative location and spacing of SNPs in kilobases within the 161-kb region containing PTPN1. Note that this does not have a uniform scale.

TABLE 1  
Association analysis of PTPN1 SNPs with  $S_i$

SNP	Alleles	MAF	Phenotypic mean $\pm$ SD			<i>P</i>
			1/1	1/2	2/2	
rs2904268	G/C	0.18	2.08 $\pm$ 1.89	2.21 $\pm$ 2.01	2.35 $\pm$ 1.74	0.936
rs803742	C/T	0.32	2.18 $\pm$ 1.99	2.05 $\pm$ 1.91	2.33 $\pm$ 1.76	0.428
rs1967439	G/A	0.41	2.28 $\pm$ 1.98	2.07 $\pm$ 1.79	2.07 $\pm$ 2.17	0.203
rs4811074	C/T	0.31	2.27 $\pm$ 1.95	2.07 $\pm$ 1.87	2.09 $\pm$ 2.18	0.173
rs4811075	G/A	0.40	2.28 $\pm$ 2.02	2.08 $\pm$ 1.93	2.03 $\pm$ 2.05	0.308
rs4811077	T/C	0.12	2.16 $\pm$ 1.95	2.06 $\pm$ 2.00	2.13 $\pm$ 1.38	0.992
rs718630	A/C	0.40	2.27 $\pm$ 1.91	2.07 $\pm$ 1.88	1.86 $\pm$ 1.89	0.088
rs3787334	C/T	0.48	1.97 $\pm$ 1.86	2.04 $\pm$ 1.77	2.46 $\pm$ 2.19	<b>0.022</b>
rs2206656	G/C	0.40	2.39 $\pm$ 2.08	1.94 $\pm$ 1.75	2.01 $\pm$ 1.97	<b>0.003</b>
rs932420	C/T	0.48	1.96 $\pm$ 1.85	2.00 $\pm$ 1.76	2.41 $\pm$ 2.16	<b>0.019</b>
rs2426158	A/G	0.35	2.34 $\pm$ 2.05	1.92 $\pm$ 1.70	1.91 $\pm$ 2.29	<b>0.004</b>
rs2904269	A/C	0.48	1.98 $\pm$ 1.86	1.98 $\pm$ 1.74	2.39 $\pm$ 2.17	<b>0.028</b>
rs941798	A/G	0.48	2.04 $\pm$ 2.06	1.97 $\pm$ 1.67	2.62 $\pm$ 2.27	<b>0.010</b>
rs1570179	C/T	0.38	2.32 $\pm$ 2.02	1.99 $\pm$ 1.78	2.00 $\pm$ 2.07	<b>0.020</b>
rs3787345	T/C	0.40	2.34 $\pm$ 2.07	1.96 $\pm$ 1.78	1.98 $\pm$ 2.00	<b>0.008</b>
rs2038526	C/T	0.38	2.34 $\pm$ 2.04	2.00 $\pm$ 1.80	2.01 $\pm$ 2.05	<b>0.025</b>
rs1885177	A/C	0.48	1.98 $\pm$ 1.85	2.00 $\pm$ 1.77	2.42 $\pm$ 2.17	<b>0.028</b>
rs754118	C/T	0.38	2.34 $\pm$ 2.04	2.00 $\pm$ 1.79	2.01 $\pm$ 2.07	<b>0.028</b>
rs3215684	T/O	0.39	2.30 $\pm$ 2.04	1.95 $\pm$ 1.80	2.01 $\pm$ 2.07	<b>0.044</b>
rs968701	G/A	0.47	1.97 $\pm$ 1.85	2.02 $\pm$ 1.77	2.40 $\pm$ 2.14	<b>0.043</b>
rs2282147	C/T	0.38	2.29 $\pm$ 1.99	1.99 $\pm$ 1.79	2.00 $\pm$ 2.06	<b>0.027</b>
rs718049	T/C	0.39	2.30 $\pm$ 2.04	1.97 $\pm$ 1.78	1.93 $\pm$ 1.94	<b>0.020</b>
rs718050	G/A	0.39	2.31 $\pm$ 2.06	1.97 $\pm$ 1.77	2.00 $\pm$ 2.03	<b>0.026</b>
rs3787348	G/T	0.48	2.03 $\pm$ 2.04	1.94 $\pm$ 1.59	2.54 $\pm$ 2.22	<b>0.006</b>
rs914460	T/C	0.38	2.31 $\pm$ 2.02	1.99 $\pm$ 1.78	2.00 $\pm$ 2.06	<b>0.015</b>
rs2426164	A/G	0.41	2.33 $\pm$ 2.04	1.98 $\pm$ 1.82	2.08 $\pm$ 2.07	<b>0.018</b>
1484insG	O/G	0.06	2.10 $\pm$ 1.88	2.22 $\pm$ 1.74	2.18 $\pm$ 1.06	<b>0.004</b>
rs1060402	G/A	0.49	1.95 $\pm$ 1.74	2.09 $\pm$ 1.91	2.34 $\pm$ 2.08	0.117
rs914458	C/G	0.25	2.15 $\pm$ 2.05	2.12 $\pm$ 1.85	2.06 $\pm$ 1.56	0.655

Only nondiabetic subjects were included in this analysis. Boldface indicates statistical significance. MAF, minor allele frequency.

respectively. The SNPs were chosen to tag all common haplotypes ( $\geq 10\%$  frequency) and  $>85\%$  of the variation in the LD block and, in addition, includes 1484insG. The Tables show the common ( $>1\%$ ) haplotypes, their frequency, *P* values for association under different models of inheritance, the mean trait values for the different haplotype combinations, and the effect of the specific haplotype on the trait. The haplotype ACTTCAG0 is significantly associated with lower  $S_i$  (e.g., greater insulin resistance) and higher fasting glucose in the dominant model, and the haplotype GTCCTGT0 is significantly associated with higher  $S_i$  (e.g., greater insulin sensitivity) and lower fasting glucose in the additive and recessive models. The remaining haplotype, ATCCTGG0, shows evidence of association with  $S_i$  under the recessive model, but this result is driven by a relatively small number of homozygotes for this haplotype ( $\sim 1\%$  subjects) and should be viewed cautiously. This haplotype has no evidence of association with fasting glucose, so we have tentatively designated ATCCTGG0 as neutral in its effect. It is noteworthy that the ACTTCAG0 and GTCCTGT0 haplotypes have independently been shown to be associated with type 2 diabetes risk and protection, respectively (10).

Protein tyrosine phosphatase-1B (PTP-1B) is a ubiquitously expressed phosphatase that dephosphorylates the phosphotyrosine residues of the active insulin receptor, disrupting the insulin signaling pathway. The PTPN1 gene has been the object of several searches for coding variants that would alter gene expression or function (7–9), but

conventional coding variants of PTPN1 are few in number and have low heterozygosities (7,8).

We evaluated measures of glucose homeostasis for association with SNPs located within the genomic region containing PTPN1. Hispanic-American populations from the IRASFS formed the basis of this analysis. Each measure of glucose homeostasis explored represents a unique component of this balanced system.

The 35 SNPs (Fig. 1) genotyped in this study fall into two discrete LD blocks determined by *D'* values (data supplement). A small block 5' to the PTPN1 gene is formed from SNPs rs1967439 to rs4811075 and a larger block from rs4811077 to rs1060402 containing PTPN1. Within the larger LD block encompassing the PTPN1 coding sequence, there is consistent, significant evidence of association with  $S_i$  and fasting glucose (Tables 1 and 2). These results are consistent with the proposed role of PTP-1B in the cell: regulation of insulin receptor activity by dephosphorylation of the insulin receptor kinase domain would lead to different levels of insulin sensitivity. Fasting glucose levels should reflect the body's response to the efficiency of the insulin signaling pathway, with higher fasting glucose levels in subjects with PTPN1 alleles associated with lower  $S_i$ . This is indeed what is observed (Tables 1 and 2). Evaluation of model-dependent (i.e., dominant, additive, and recessive) analyses did not suggest a specific mode of inheritance for the single PTPN1 SNPs and either phenotype (data not shown).

In contrast to  $S_i$  and fasting glucose, there is little to no

TABLE 2  
Association analysis of PTPN1 SNPs with fasting glucose (milligrams per deciliter)

SNP	Alleles	MAF	Phenotypic mean $\pm$ SD			<i>P</i>
			1/1	1/2	2/2	
rs2904268	G/C	0.18	93.72 $\pm$ 9.85	92.96 $\pm$ 9.42	91.37 $\pm$ 9.17	0.719
rs803742	C/T	0.32	93.32 $\pm$ 9.40	93.53 $\pm$ 9.95	94.46 $\pm$ 11.0	0.586
rs1967439	G/A	0.41	93.27 $\pm$ 9.76	93.60 $\pm$ 9.32	93.05 $\pm$ 9.84	0.338
rs4811074	C/T	0.31	93.40 $\pm$ 9.78	93.87 $\pm$ 9.69	93.01 $\pm$ 9.05	0.416
rs4811075	G/A	0.40	93.52 $\pm$ 9.58	93.55 $\pm$ 9.79	93.07 $\pm$ 9.27	0.658
rs4811077	T/C	0.12	93.36 $\pm$ 9.55	92.79 $\pm$ 9.73	96.61 $\pm$ 12.9	0.576
rs718630	A/C	0.40	92.32 $\pm$ 9.30	94.35 $\pm$ 10.1	93.68 $\pm$ 8.91	<b>0.024</b>
rs3787334	C/T	0.48	93.93 $\pm$ 9.64	93.80 $\pm$ 9.81	92.41 $\pm$ 9.86	<b>0.001</b>
rs2206656	G/C	0.40	92.33 $\pm$ 9.59	94.21 $\pm$ 9.87	94.03 $\pm$ 9.20	<b>0.006</b>
rs932420	C/T	0.48	94.07 $\pm$ 9.41	93.82 $\pm$ 9.70	92.64 $\pm$ 9.89	<b>0.010</b>
rs2426158	A/G	0.35	92.21 $\pm$ 9.62	94.97 $\pm$ 9.68	93.79 $\pm$ 8.51	<b>0.006</b>
rs2904269	A/C	0.48	93.84 $\pm$ 9.51	93.84 $\pm$ 9.71	92.63 $\pm$ 9.94	<b>0.018</b>
rs941798	A/G	0.48	94.27 $\pm$ 9.81	94.15 $\pm$ 9.88	91.22 $\pm$ 9.13	<b>0.001</b>
rs1570179	C/T	0.38	92.03 $\pm$ 9.43	94.60 $\pm$ 10.0	94.14 $\pm$ 9.34	<b>&lt;0.001</b>
rs3787345	T/C	0.40	92.18 $\pm$ 9.63	94.33 $\pm$ 9.75	94.26 $\pm$ 9.67	<b>0.001</b>
rs2038526	C/T	0.38	92.10 $\pm$ 9.52	94.54 $\pm$ 9.92	93.47 $\pm$ 9.06	<b>0.001</b>
rs1885177	A/C	0.48	93.85 $\pm$ 9.58	93.87 $\pm$ 9.66	92.42 $\pm$ 9.75	<b>0.010</b>
rs754118	C/T	0.38	91.94 $\pm$ 9.31	94.59 $\pm$ 10.0	93.96 $\pm$ 9.37	<b>&lt;0.001</b>
rs3215684	T/O	0.39	92.14 $\pm$ 9.53	94.71 $\pm$ 9.88	93.96 $\pm$ 9.37	<b>0.001</b>
rs968701	G/A	0.47	93.83 $\pm$ 9.61	93.85 $\pm$ 9.61	92.44 $\pm$ 9.74	<b>0.008</b>
rs2282147	C/T	0.38	92.07 $\pm$ 9.37	94.54 $\pm$ 10.0	93.95 $\pm$ 9.30	<b>&lt;0.001</b>
rs718049	T/C	0.39	92.19 $\pm$ 9.53	94.61 $\pm$ 9.90	93.75 $\pm$ 9.28	<b>&lt;0.001</b>
rs718050	G/A	0.39	92.22 $\pm$ 9.55	94.70 $\pm$ 10.0	93.66 $\pm$ 9.24	<b>&lt;0.001</b>
rs3787348	G/T	0.48	94.19 $\pm$ 9.65	93.93 $\pm$ 9.83	91.72 $\pm$ 9.38	<b>0.004</b>
rs914460	T/C	0.38	92.03 $\pm$ 9.44	94.55 $\pm$ 9.87	93.95 $\pm$ 9.30	<b>&lt;0.001</b>
rs2426164	A/G	0.41	92.05 $\pm$ 9.36	95.04 $\pm$ 10.1	93.51 $\pm$ 9.32	<b>&lt;0.001</b>
1484insG	O/G	0.06	93.59 $\pm$ 9.78	91.41 $\pm$ 8.54	90.75 $\pm$ 4.56	<b>0.013</b>
rs1060402	G/A	0.49	93.53 $\pm$ 9.08	93.86 $\pm$ 9.88	92.83 $\pm$ 9.94	<b>0.029</b>
rs914458	C/G	0.25	93.72 $\pm$ 9.83	93.13 $\pm$ 9.59	96.27 $\pm$ 10.3	0.449

Only nondiabetic subjects were included in this analysis. Boldface indicates statistical significance. MAF, minor allele frequency.

evidence of association of PTPN1 SNPs with AIR (Table 3). This result is again consistent with the cellular role of PTP-1B. AIR, a measure of  $\beta$ -cell function, would not be directly affected by alterations in the availability or function of the PTP-1B protein. This result would also suggest that  $\beta$ -cell function, as reflected in AIR, does not directly influence insulin resistance. We observed no significant evidence of association between PTPN1 SNPs and type 2 diabetes as a qualitative trait in this study (data not shown). The study design of large families with a relatively small number of type 2 diabetic subjects (16%,  $n = 129$ ), however, has limited power to test for association with type 2 diabetes.

We also evaluated two previously described SNPs within the coding region of PTPN1 (7,8). The SNP identified by Mok et al. (8) (981 C $\rightarrow$ T) had a minor allele frequency of  $\sim$ 4% in the Hispanic populations and no homozygotes for the T allele were found. There is no

evidence of association with any of the six measures of glucose homeostasis examined in this study (data not shown). The coding mutation reported by Echwald et al. (7) was not observed, i.e., it was found to be monomorphic, in our Hispanic populations. It seems unlikely that these SNPs are responsible for the associations that we observe with glucose homeostasis phenotypes. In addition, we have evaluated whether the 1484insG SNP, located in the 3' UTR, is the trait-defining variant. This SNP is associated with  $S_i$  ( $P = 0.004$ ) and fasting glucose ( $P = 0.013$ ), as are other SNPs in the haplotype block. However, the phenotypic means for  $S_i$  and fasting glucose do not present consistent evidence to confirm the association of the presence of the G allele with insulin resistance, i.e., individuals with the insertion have higher  $S_i$  (are less insulin resistant), and there is no clear interpretation of the fasting glucose data. This result could be due to the low minor allele frequency (6%), resulting in only two

TABLE 4  
PTPN1 haplotype association with  $S_i$  in Hispanic subjects in the IRASFS

Haplotype	Frequency	Disposition	<i>P</i> for each model			Haplo-genotype mean $S_i$		
			Dominant	Additive	Recessive	+/+*	+/-†	-/-‡
GTCCTGTO	0.43	Protective: higher $S_i$	0.28	<b>0.00098</b>	<b>0.00024</b>	2.45 $\pm$ 2.46	1.81 $\pm$ 1.41	1.82 $\pm$ 1.97
ACTTCAGO	0.39	Risk: lower $S_i$	<b>0.0064</b>	0.11	0.47	1.74 $\pm$ 2.00	1.84 $\pm$ 1.58	2.20 $\pm$ 2.06
ATCCTGGO	0.10	Neutral	0.81	0.96	<b>0.00004</b>	1.06 $\pm$ 0.94	1.91 $\pm$ 1.93	2.00 $\pm$ 1.81

Data are means  $\pm$  SD. Boldface indicates statistical significance. \*Two copies of the haplotype of interest; †one copy of the haplotype of interest and any other haplotype; ‡any other haplotype combination.

TABLE 3  
Association analysis of PTPN1 SNPs with AIR

SNP	Alleles	MAF	Phenotypic mean $\pm$ SD			<i>P</i>
			1/1	1/2	2/2	
rs2904268	G/C	0.18	740 $\pm$ 594	817 $\pm$ 703	618 $\pm$ 553	0.311
rs803742	C/T	0.32	756 $\pm$ 605	745 $\pm$ 618	738 $\pm$ 674	0.971
rs1967439	G/A	0.41	740 $\pm$ 648	724 $\pm$ 531	849 $\pm$ 785	0.918
rs4811074	C/T	0.31	743 $\pm$ 651	722 $\pm$ 513	929 $\pm$ 828	0.439
rs4811075	G/A	0.40	762 $\pm$ 664	734 $\pm$ 560	857 $\pm$ 770	0.435
rs4811077	T/C	0.12	745 $\pm$ 606	804 $\pm$ 701	421 $\pm$ 298	<b>0.012</b>
rs718630	A/C	0.40	776 $\pm$ 668	744 $\pm$ 564	740 $\pm$ 702	0.374
rs3787334	C/T	0.48	755 $\pm$ 663	787 $\pm$ 634	686 $\pm$ 560	0.327
rs2206656	G/C	0.40	734 $\pm$ 597	795 $\pm$ 652	718 $\pm$ 645	0.361
rs932420	C/T	0.48	755 $\pm$ 663	796 $\pm$ 636	705 $\pm$ 594	0.356
rs2426158	A/G	0.35	737 $\pm$ 586	692 $\pm$ 553	719 $\pm$ 576	0.427
rs2904269	A/C	0.48	754 $\pm$ 661	781 $\pm$ 626	714 $\pm$ 603	0.533
rs941798	A/G	0.48	734 $\pm$ 689	766 $\pm$ 576	756 $\pm$ 644	0.593
rs1570179	C/T	0.38	772 $\pm$ 634	764 $\pm$ 629	670 $\pm$ 595	0.214
rs3787345	T/C	0.40	766 $\pm$ 643	771 $\pm$ 613	740 $\pm$ 676	0.541
rs2038526	C/T	0.38	766 $\pm$ 631	775 $\pm$ 640	662 $\pm$ 588	0.200
rs1885177	A/C	0.48	750 $\pm$ 663	787 $\pm$ 631	724 $\pm$ 602	0.548
rs754118	C/T	0.38	778 $\pm$ 636	753 $\pm$ 610	668 $\pm$ 596	0.171
rs3215684	T/O	0.39	775 $\pm$ 645	774 $\pm$ 632	668 $\pm$ 596	0.238
rs968701	G/A	0.47	751 $\pm$ 664	783 $\pm$ 633	717 $\pm$ 606	0.464
rs2282147	C/T	0.38	774 $\pm$ 638	760 $\pm$ 629	666 $\pm$ 591	0.194
rs718049	T/C	0.39	775 $\pm$ 644	777 $\pm$ 631	667 $\pm$ 582	0.125
rs718050	G/A	0.39	773 $\pm$ 648	766 $\pm$ 627	661 $\pm$ 580	0.140
rs3787348	G/T	0.48	725 $\pm$ 682	780 $\pm$ 589	744 $\pm$ 642	0.407
rs914460	T/C	0.38	774 $\pm$ 635	765 $\pm$ 624	666 $\pm$ 591	0.183
rs2426164	A/G	0.41	764 $\pm$ 646	726 $\pm$ 619	721 $\pm$ 667	0.498
1484insG	O/G	0.06	735 $\pm$ 605	937 $\pm$ 692	637 $\pm$ 71	<b>&lt;0.001</b>
rs1060402	G/A	0.49	785 $\pm$ 694	767 $\pm$ 618	707 $\pm$ 590	0.788
rs914458	C/G	0.25	771 $\pm$ 619	732 $\pm$ 594	614 $\pm$ 500	0.547

Only nondiabetic subjects were included in this analysis. Boldface indicates statistical significance. MAF, minor allele frequency.

homozygotes for the insertion. From this data, we cannot include or exclude this SNP as a contributor to the overall trait differences. It is noteworthy that in a study of Caucasian-American type 2 diabetic subjects and nondiabetic control subjects, the insertion G allele is not associated with diabetes risk (10).

Results of haplotype analysis are consistent with the single SNP results and the pattern of haplotypic association of PTPN1 SNPs with type 2 diabetes in Caucasians (10). For the eight-SNP haplotype, there is one common type 2 diabetes risk haplotype, ACTTCAGO, and one common type 2 diabetes protective haplotype, GTCCTGTO, in Caucasians. In the IRASFS Hispanics, a similar pattern is observed: the type 2 diabetes risk haplotype is common (39% of the chromosomes) and significantly associated with lower  $S_i$  and higher fasting glucose. Likewise, the common type 2 diabetes pro-

tective haplotype (43% of the chromosomes) is significantly associated with higher  $S_i$  and lower fasting glucose. The pattern and direction of associations is also consistent with single SNP results.

The purpose of this study was to evaluate the relationship between SNPs in the genomic region containing PTPN1 and quantitative measures of glucose homeostasis, including  $S_i$ , glucose effectiveness ( $S_g$ ), AIR, disposition index, fasting glucose, and fasting insulin. The results demonstrate a consistent pattern of association that was dictated by the LD structure within the region. Measures of glucose homeostasis,  $S_i$  and fasting glucose, which are centered on the insulin signaling pathway (a major target of PTP-1B) were significantly associated with SNPs located within the LD block containing PTPN1. Although the high degree of LD could be masking the causal variant, all

TABLE 5  
PTPN1 haplotype association with fasting glucose in Hispanic subjects in the IRASFS

Haplotype	Frequency	Disposition	<i>P</i> for each model			Haplo-genotype mean fasting glucose		
			Dominant	Additive	Recessive	+/+*	+/-†	-/-‡
GTCCTGTO	0.43	Protective: lower fasting glucose	0.28	<b>0.018</b>	<b>0.0015</b>	100.24 $\pm$ 30.76	103.76 $\pm$ 27.55	108.52 $\pm$ 37.08
ACTTCAGO	0.39	Risk: higher fasting glucose	<b>0.00007</b>	0.35	0.58	112.15 $\pm$ 43.20	105.19 $\pm$ 29.75	100.50 $\pm$ 27.96
ATCCTGGO	0.10	Neutral	0.21	0.87	0.17	105.83 $\pm$ 37.59	105.62 $\pm$ 30.28	103.74 $\pm$ 30.82

Data are means  $\pm$  SD. Boldface indicates statistical significance. \*Two copies of the haplotype of interest; †one copy of the haplotype of interest and any other haplotype; ‡any other haplotype combination.

TABLE 6  
Characteristics of Hispanic IRASFS participants

	<i>n</i>	Mean ± SD	Median
<i>N</i>	811		
<b>Demographic</b>			
Age (years)	719	42.2 ± 14.5	40.8
BMI (kg/m <sup>2</sup> )	714	29.15 ± 6.16	28.39
Female sex	57%	—	—
Diabetic	129	—	—
<b>Glucose homeostasis</b>			
<i>S<sub>i</sub></i> (MINMOD)	583	2.13 ± 1.92	1.61
<i>S<sub>g</sub></i> (MINMOD)	583	0.021 ± 0.009	0.020
Disposition index (AIR × <i>S<sub>i</sub></i> )	583	1,318 ± 1,271	995
Fasting glucose (mg/dl)	616	93.4 ± 9.70	92.0
Fasting insulin (μU/ml)	618	15.5 ± 11.1	13.0
AIR (μU/ml)	583	758 ± 625	598

SNPs within the LD block show significant associations, and the magnitude of the association suggests that there is a causal variant or combination of variants that account for these associations. It is noteworthy that we have observed remarkably similar results in an evaluation of PTPN1 SNPs in studies of Caucasian-American type 2 diabetic case subjects and nondiabetic control subjects (10).

## RESEARCH DESIGN AND METHODS

The study design, recruitment, and phenotyping for IRASFS have been described in detail elsewhere (11). Briefly, the IRASFS is designed to identify the genetic bases of insulin resistance and visceral adiposity, which are important risk factors for the development of type 2 diabetes and atherosclerosis. Subjects in the study have been recruited from clinical centers in San Luis Valley, Colorado (*n* = 494) (a rural Hispanic population), and San Antonio, Texas (*n* = 317) (an urban Hispanic population). Data from 55 pedigrees encompassing all 811 individuals were analyzed in this study. Diabetic subjects were not included in the analyses of glucose homeostasis measures. The clinical examination included an insulin-modified frequently sampled intravenous glucose tolerance test using the reduced sampling protocol (12) to compute glucose homeostasis measures, height, weight, and waist and hip circumferences and computed tomography to estimate visceral and subcutaneous fat, fasting blood draw, and medical history interview. Table 6 provides a descriptive summary of the primary phenotypes.

**SNP selection and genotyping.** SNPs were selected from the dbSNP database, with the exception of the published SNP, 1484insG (9). Those SNPs with available frequency information were preferentially selected from the database. The majority of the SNPs in this study had a minor allele frequency >0.20. Genotyping was performed on the Sequenom MassArray Genotyping System using methods previously described (13). Discordance between blind duplicate samples included in the genotyping was <0.2%. All 35 SNPs were genotyped on all 811 subjects.

Initially, each of the PTPN1 SNPs evaluated was examined for Mendelian inconsistencies in their genotypes using PedCheck (14). Any genotypes inconsistent with Mendelian inheritance were converted to missing. This resulted in zeroing of 129 of the 40,320 genotypes generated across the 35 SNPs. Subsequently, maximum-likelihood estimates of allele frequencies were computed using the largest set of unrelated individuals and then tested for departures from Hardy-Weinberg proportions. To test for an association between individual SNPs and each trait, a series of GEE1 (15) was computed. Familial correlation was accounted for by using a sandwich estimator of the variance and exchangeable correlation. The two-degrees-of-freedom overall test of genotypic association was the principal analysis method. Tests reported here were computed adjusting for age, sex, recruitment center (San Antonio, Texas, and San Luis Valley, Colorado), and BMI. When necessary, quantitative traits were transformed to best approximate the distributional assumptions of the test (i.e., link function) and to minimize the heterogeneity of the variance. Approximately 16% of the subjects had a diagnosis of type 2 diabetes. Type 2 diabetic subjects were excluded from the analysis of the glucose homeostasis traits. A *P* value of <0.05 was considered significant.

To test for a haplotypic association between the glucose homeostasis traits and the PTPN1 polymorphisms, a weighted GEE1 analysis was computed as

described above, with the weights being the probability for each possible haplo-genotype for an individual. Specifically, we computed the expectation-maximization algorithm estimates for haplotype frequencies from the family data using the software ZAPLO (16). To reduce the complexity of the problem, ZAPLO assumes zero recombination between markers. Using the expectation-maximization-based estimates, ZAPLO outputs all possible haplo-genotypes (i.e., haplotype combinations) for each individual. These data are input into PROFILER (17) to compute the joint probability distribution for the haplotypes of individuals within the pedigree. This joint probability distribution is then used to estimate the probability of each haplotype pair combination (haplo-genotype) possible for each individual, conditional on the family data. Each individual enters into the GEE1 analysis once for each haplo-genotype possibility, weighted by the haplo-genotype probability. Thus, the weight for each individual sums to 1. The weighted GEE1 analyses were completed as above using the same transformations and sandwich estimator of the variance to account for the within cluster correlation. Linear contrasts for the three a priori genetic models (i.e., dominant, additive, and recessive) in each of the primary haplotypes (5%) were computed.

The pairwise LD statistic *D'* was calculated using the SNP-Analysis software package (<http://www.fhcr.org/labs/kruglyak/Downloads>). A graphical summary of these LD values was generated using the GOLD software package.

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