

# Association of Protein Tyrosine Phosphatase 1B Gene Polymorphisms With Type 2 Diabetes

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The PTPN1 gene codes for protein tyrosine phosphatase 1B (PTP1B) (EC 3.1.3.48), which negatively regulates insulin signaling by dephosphorylating the phosphotyrosine residues of the insulin receptor kinase activation segment. PTPN1 is located in 20q13, a genomic region linked to type 2 diabetes in multiple genetic studies. Surveys of the gene have previously identified only a few uncommon coding single nucleotide polymorphisms (SNPs). We have carried out a detailed association analysis of 23 noncoding SNPs spanning the 161-kb genomic region, which includes the PTPN1 gene. These SNPs have been assessed for association with type 2 diabetes in two independently ascertained collections of Caucasian subjects with type 2 diabetes and two control groups. Association is observed between multiple SNPs and type 2 diabetes. The most consistent evidence for association occurred with SNPs spanning the 3' end of intron 1 of PTPN1 through intron 8 (*P* values ranging from 0.043 to 0.004 in one case-control set and 0.038–0.002 in a second case-control set). Analysis of the combined case-control data increased the evidence of SNP association with type 2 diabetes (*P* = 0.005–0.0016). All of the associated SNPs lie in a single 100-kb haplotype block that encompasses the PTPN1 gene. Analysis of haplotypes indicates a significant difference between haplotype frequencies in type 2 diabetes case and control subjects (*P* = 0.0035–0.0056), with one common haplotype (36%) contributing strongly to the evidence for association with type 2 diabetes. Odds ratios calculated from single SNP or haplotype data are in the proximity of 1.3. Haplotype-based calculation of population-attributable risk (PAR) results in an estimated PAR of 17–20% based on different models and

assumptions. These results suggest that PTPN1 is a significant contributor to type 2 diabetes susceptibility in the Caucasian population. This risk is likely due to noncoding polymorphisms. *Diabetes* 53:3007–3012, 2004

**M**ultiple genetic studies have been carried out that link human chromosome 20q12–13.1 to type 2 diabetes (1–5). The linkage evidence has led investigators to search for type 2 diabetes susceptibility genes in this genomic region. Our laboratory has carried out analysis of specific genes (6–8) and developed high-resolution physical maps of the region (9–11). In an association analysis of genetic markers, Price et al. (12) identified three regions of type 2 diabetes susceptibility. Within one of these type 2 diabetes-associated regions is a positional candidate gene, PTPN1, that codes for the protein tyrosine phosphatase 1B (PTP1B) protein.

PTP1B is a ubiquitously expressed phosphatase that dephosphorylates phosphotyrosine residues of the active insulin receptor, disrupting the insulin signaling pathway (13,14). PTP1B-deficient mice show increased insulin sensitivity and resistance to diet-induced obesity (15,16). Both the function and genomic location are consistent with a role in type 2 diabetes, possibly mediated through a contribution to insulin resistance. Thus, PTPN1 represents a promising positional candidate gene for analysis in type 2 diabetes and associated phenotypes.

The PTPN1 gene has been the object of several searches for coding variants that would alter gene expression or function (17–19). Coding variants of PTPN1 are uncommon (17–19). Other polymorphisms may contribute to diabetes phenotypes. To examine the association of PTPN1 variants with type 2 diabetes, a genetic analysis was performed with 23 single nucleotide polymorphisms (SNPs) covering the 161-kb region encoding PTPN1 and the 5' and 3' genomic sequences. Evidence for association of these polymorphisms with type 2 diabetes was assessed in two independently ascertained case-control populations.

## RESULTS

The PTPN1 genomic sequence consists of 10 exons spanning 74 kb with a large first intron of >54 kb (20). We identified and evaluated 23 SNPs located throughout the genomic region, as shown in Fig. 1. All of the SNPs with

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DHS, Diabetes Heart Study; ESRD, end-stage renal disease; PAR, population-attributable risk; PTP1B, protein tyrosine phosphatase 1B; SNP, single nucleotide polymorphism.

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FIG. 1. Genomic map of PTPN1 gene with location 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.

minor allele frequencies  $>0.2$  were found in noncoding regions. Average SNP density was 1 SNP per 7.0 kb, with the largest gap between SNPs being 29.4 kb and the smallest being 68 bp.

Initially SNPs were genotyped in a collection of Caucasian type 2 diabetes with end-stage renal disease (ESRD): 300 type 2 diabetic ESRD case subjects and 310 Caucasian control subjects. All SNPs were consistent with Hardy-Weinberg equilibrium, except rs4811078 ( $P = 0.01$ ) and rs2426158 ( $P = 0.04$ ). The initial association analysis with SNPs, shown in online appendix Table 1 (available from <http://diabetes.diabetesjournals.org>), revealed evidence for an association between eight SNPs and type 2 diabetes in the type 2 diabetic ESRD subjects (rs2206656, rs1570179, rs3787345, rs754118, rs3215684, rs2282147, rs718049, and 1484insG;  $P = 0.015$ – $0.048$ ). Results for two other SNPs (rs718050,  $P = 0.058$ , and rs718630,  $P = 0.08$ ) were suggestive of association. Single marker tests of genotypic association yielded similar results under a recessive model for each of the above eight SNPs ( $P = 0.007$ – $0.048$ ), with the exception of 1484insG (data not shown). Although two SNPs (rs2426158 and rs718050) did not exhibit allelic association with type 2 diabetes, there was evidence of genotypic association ( $P = 0.019$  and  $0.006$ , respectively; data not shown).

Based on evidence of association of PTPN1 SNPs with type 2 diabetes in the type 2 diabetic ESRD case-control subjects, we tested these results for replication in a second, independently ascertained case-control group consisting of 275 Diabetes Heart Study (DHS) type 2 diabetic case subjects and 200 Caucasian control subjects. The results of the association analysis in this case-control group are shown in online appendix Table 2. SNPs rs941798, rs1570179, rs2282147, rs718049, rs718050, and rs3787348 also have evidence of significant association with type 2 diabetes ( $P = 0.047$ – $0.0024$ ) and with several SNPs trending toward association.

When data from both case-control populations were pooled and analyzed for association (Table 1) there was increased evidence of association for multiple SNPs across the PTPN1 gene. Of 11 SNPs in map order, 7 show evidence for association with type 2 diabetes ( $P = 0.0053$ – $0.0016$ ): rs941798 to rs3787348 (rs941798, rs3787345, rs754118, rs2282147, rs718049, rs718050, and rs3787348). The SNPs from rs941798 to rs3787348 cover PTPN1 from the end of exon 1 through distal exon 8, with a block of four associated SNPs (rs2282147 to rs3787348) that span the distal end of intron 7 through distal intron 8, providing the most consistent evidence for association with type 2 diabetes for any group of contiguous SNPs.

Linkage disequilibrium and the haplotype structure of PTPN1 were investigated to determine whether specific SNP haplotypes were associated with type 2 diabetes. Inter-SNP  $D'$  statistics were calculated for the PTPN1 gene region (Fig. 2). With  $D' > 0.8$  defined as a criteria for high disequilibrium, the majority of SNPs lie within one block of  $\sim 100$  kb. This block starts with SNP rs718630 in the promoter region, ending with 1484insG SNP in the 3' untranslated region.

Haplotype frequencies were estimated and association analyses performed with respect to type 2 diabetes in the two case-control populations. Shown in Table 2 are the

TABLE 1  
Distribution of alleles for the 23 SNPs in pooled Caucasian case and control populations

SNP	Alleles	Frequency		P
		Case subjects (n = 575)	Control subjects (n = 510)	
rs2904268	C/G	0.34/0.66	0.35/0.64	0.37
rs803742	T/C	0.44/0.56	0.45/0.55	0.6
rs1967439	A/G	0.33/0.67	0.30/0.69	0.25
rs718630	C/A	0.43/0.57	0.39/0.61	0.043
rs4811078	T/C	0.12/0.87	0.15/0.84	0.037
rs2206656	C/G	0.43/0.57	0.37/0.62	0.01
rs932420	C/T	0.50/0.48	0.47/0.53	0.13
rs3787335	G/T	0.10/0.89	0.09/0.91	0.28
rs2426158	G/A	0.29/0.70	0.26/0.74	0.078
rs2904269	A/C	0.51/0.48	0.47/0.53	0.71
rs941798	G/A	0.41/0.59	0.47/0.52	0.0018
rs1570179	T/C	0.40/0.59	0.34/0.66	0.57
rs3787345	C/T	0.44/0.55	0.38/0.62	0.0042
rs1885177	A/C	0.52/0.48	0.47/0.52	0.12
rs754118	T/C	0.41/0.59	0.34/0.65	0.0016
rs3215684	O/T	0.40/0.59	0.34/0.66	0.06
rs968701	G/A	0.52/0.48	0.48/0.52	0.16
rs2282147	T/C	0.40/0.59	0.34/0.65	0.0028
rs718049	C/T	0.43/0.57	0.36/0.63	0.002
rs718050	A/G	0.42/0.58	0.36/0.64	0.0053
rs3787348	T/G	0.41/0.59	0.47/0.53	0.002
1484 ins G	G/O	0.067/0.93	0.096/0.90	0.013
rs914458	G/C	0.30/0.70	0.30/0.70	0.99

results from HAPLO.SCORE evaluating eight SNP haplotypes within the PTPN1 gene. The eight SNPs (rs941798, rs3787345, rs754118, rs2282147, rs718049, rs718050, rs3787348, and 1484insG) were chosen to tag all common haplotypes ( $\geq 10\%$  frequency) and  $>85\%$  of the variation in

the linkage disequilibrium block and, in addition, include 1484insG (previously proposed as a functional SNP) (17). The analysis results are summarized as the specific haplotype, the haplotype frequency in case and control subjects, and a score for each haplotype (HAP-SCORE) in which a negative sign shows a higher proportion of this haplotype in control subjects and a positive sign indicates a higher proportion in type 2 diabetic case subjects. Also reported are haplotype-specific empiric P values for significance of the HAP-SCORE. An overall P value is reported for the significance of the difference between type 2 diabetic case and control subjects. Results of analyses of the individual case-control populations and the combined analysis are shown.

Six haplotypes are estimated in each of the three analyses. The haplotype frequency differences are significant for the type 2 diabetic ESRD case-control group ( $P = 0.041$ ) and approaching significant for the DHS case-control group ( $P = 0.055$ ). When the two populations are combined, an overall statistically significant difference is observed between type 2 diabetic case and control subjects ( $P = 0.0035$ ). A common risk haplotype, ACTTCAG0, is most strongly associated with type 2 diabetes, and a second common haplotype, GTCCTGT0, is most common in control subjects. In addition, a second less common haplotype (7% overall, ATCCTGG0) shows some evidence for association with type 2 diabetes.

DISCUSSION

The PTPN1 gene is located in 20q13 and codes for PTP1B, a protein well documented as having a role in the regulation of insulin signaling. This obvious functional link has led investigators to search for allelic variants in the PTPN1 gene. Coding variants are uncommon. Di Paola et al. (17) identified a 3' untranslated region polymorphism of  $\sim 7\%$

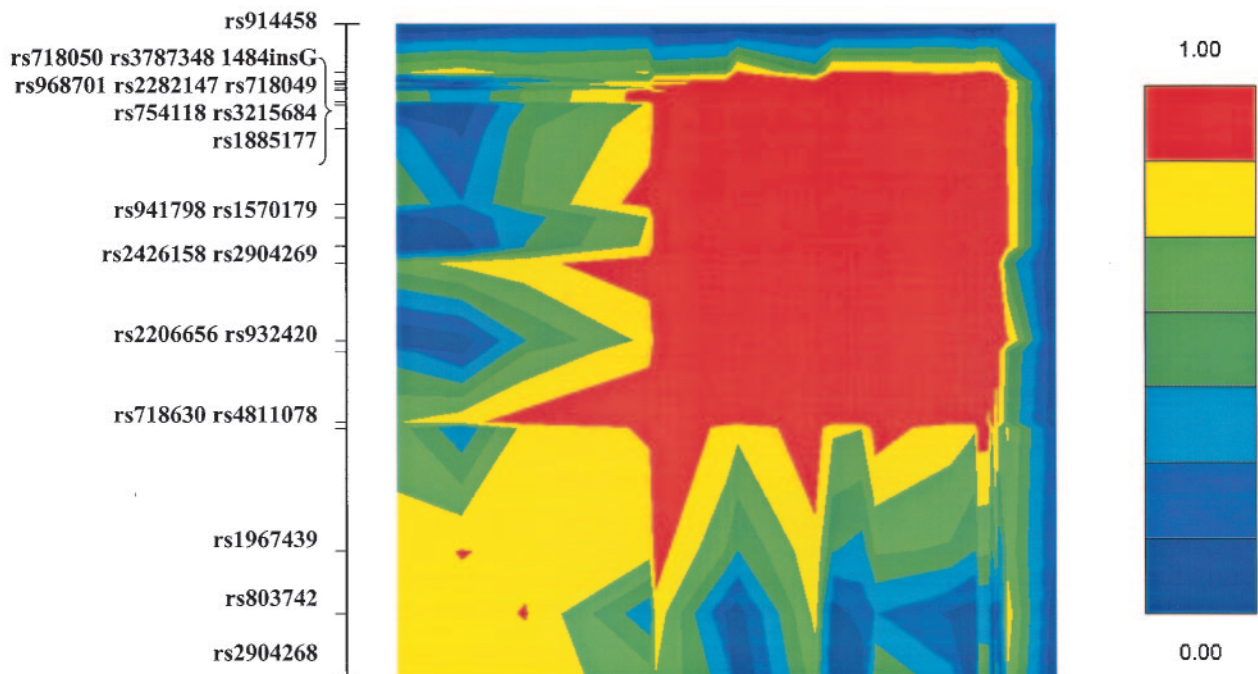


FIG. 2. Marker-to-marker D' plot for the PTPN1 SNPs. Inter-SNP D' values are graphically represented using the graphical overview of linkage disequilibrium (GOLD) package, which generates the color coded plot of the pairwise disequilibrium statistics with horizontal and vertical axes that are scaled according to the inter-SNP distances (32).

TABLE 2

Haplotype analysis using HAPLO.SCORE with eight SNP (rs941798, rs3787345, rs754118, rs2282147, rs718049, rs718050, rs3787348, and 1484insG) PTPN1 haplotypes

Haplotype	Haplotype frequency in case subjects	Haplotype frequency in control subjects	Overall haplotype frequency	HAP-Score	Empirical HAP-specific <i>P</i> value	Overall <i>P</i> value
300 Caucasian type 2 diabetic-ESRD and 310 Caucasian control subjects						
GTCCTGTO	0.41	0.45	0.43	-1.87	0.057	0.041
ATCCTGGG	0.06	0.09	0.07	-2.12	0.028	
ACCCTGGO	0.02	0.02	0.02	0.49	0.470	
ACCCCAGO	0.02	0.02	0.02	0.18	0.940	
ATCCTGGO	0.09	0.07	0.08	1.4	0.175	
ACTTCAGO	0.39	0.33	0.36	2.65	0.009	
275 Caucasian DHS-type 2 diabetic and 200 Caucasian control subjects						
GTCCTGTO	0.39	0.47	0.43	-2.99	0.0023	0.055
ATCCTGGG	0.07	0.08	0.08	-1.91	0.054	
ACCCTGGO	0.01	0.02	0.02	-0.64	0.490	
ACCCCAGO	0.03	0.02	0.02	0.25	0.800	
ATCCTGGO	0.07	0.04	0.06	1.95	0.061	
ACTTCAGO	0.41	0.34	0.37	2.11	0.033	
Combined case and control populations: 575 case subjects and 510 control subjects						
GTCCTGTO	0.41	0.46	0.43	-2.99	0.002	0.003
ATCCTGGG	0.07	0.09	0.08	-1.91	0.055	
ACCCTGGO	0.013	0.02	0.02	-0.63	0.47	
ACCCCAGO	0.02	0.02	0.02	0.25	0.8	
ATCCTGGO	0.08	0.06	0.07	2.15	0.03	
ACTTCAGO	0.40	0.33	0.36	3.42	0.0004	

minor allele frequency consisting of an insertion/deletion of G at position 1484 (1484insG) of the cDNA and described evidence for association in male (but not female) subjects with higher values of insulin resistance measured by homeostasis model assessment for insulin resistance ( $P = 0.006$ ), serum triglycerides ( $P = 0.0002$ ), and total-to-HDL cholesterol ratio ( $P = 0.025$ ). Echwald et al. (18) identified a P387L variant that was found in 2.6% of type 2 diabetes-affected individuals and 1% of normal subjects that showed evidence of impaired in vitro serine phosphorylation of the PTP1B peptide. Mok et al. (19) described a relatively uncommon (5% minor allele frequency) 981C→T polymorphism in the cDNA for which they observed evidence for association with impaired glucose tolerance or type 2 diabetes in the Oji-Cree Indian tribe. This latter SNP, however, is silent, i.e., leads to no change in the coding sequence of the PTP1B protein. We have evaluated the P387L and 981C→T SNPs in our study and found them to be rare or missing and not associated with diabetes (data not shown).

In earlier association studies we observed evidence for association of the PTPN1-containing chromosomal region with type 2 diabetes in a case-control study (12). We have also observed association with  $S_i$ , a measure of insulin sensitivity (21), in large, mostly nondiabetic Hispanic families. These observations led us to evaluate the PTPN1 gene for association with type 2 diabetes using a set of SNPs covering the genomic DNA sequence. In SNP tests, we observed significant evidence of association between multiple PTPN1 SNPs and type 2 diabetes in two independently ascertained collections of type 2 diabetic case and control subjects (online appendix Tables 1 and 2). When genotypic data from the two studies are combined (Table 1), the evidence for association between PTPN1 SNPs and type 2 diabetes is stronger, with 11 SNPs showing signifi-

cant evidence for association ( $P$  values 0.043–0.0016) in the region defined by rs718630 to 1484insG. The most consistent evidence for association is with SNPs in the distal part of the gene (intron 4 rs3787345 to intron 8 rs3787348) in which six of nine SNPs show evidence of association and the other three SNPs show a trend in that direction. This pattern of association is consistent with the block of strong linkage disequilibrium identified using  $D'$  (Fig. 2).

Haplotype analysis suggests that significant association with diabetes ( $P = 0.0035$ – $0.0056$ ) is predominately due to a single common (~36%) haplotype (ACTTCAGO) in the type 2 diabetic case subjects and that a second less common haplotype may contribute risk. The pattern of association with type 2 diabetes is the same in the two independent case-control populations, and the major haplotype frequencies are virtually identical. The similarity of the overall haplotype frequency profile across the two populations strongly suggests that the haplotype frequency estimates are reproducible and that the association is nonspurious within these type 2 diabetic populations. In the haplotype association analysis the significance of differences between the case and control subjects (Table 2) is comparable in magnitude to that observed with individual SNPs. As such, construction of haplotypes does not reveal evidence of a subset of chromosomes that are more powerfully associated with diabetes than revealed by the individual SNPs. Most of the type 2 diabetes risk resides in a single haplotype, suggesting that a single SNP or unique combination of SNPs is contributing to type 2 diabetes risk. It is noteworthy that the common risk and protective haplotypes are almost completely mismatching, which makes it challenging to identify any single SNP as functional. The relatively high frequency for the major risk

haplotype (36%) is consistent with the common variant common disease hypothesis.

The evidence that multiple noncoding SNPs are associated with a complex genetic trait such as diabetes is not surprising given recent reports of similar findings with Crohn's disease (22), asthma (23), and schizophrenia (24). We have specifically evaluated whether the 1484insG SNP, a variant for which functional changes in gene expression have been proposed (17), is the trait-defining variant by assessing whether haplotypes made up of PTPN1 SNPs and containing the 1484insG allele contribute greater risk than other SNPs in the region (Table 2). Haplotypes containing 1484insG are no more strongly associated than other haplotypes, and the type 2 diabetes risk haplotype, ACTTCAG0, contains the common 0 (no G insertion) allele and not the insG allele. The insG allele is observed only in a neutral/protective haplotype. None of the other associated SNPs are within sequences that would suggest a potential functional role in the cell. We carried out extensive sequencing and found no new coding SNPs. We cannot exclude a functional role for the SNPs genotyped here, but it seems likely that the diabetes susceptibility SNP(s) has not yet been identified. The SNPs with which we observe association are surrogates for the diabetes susceptibility SNPs.

We have calculated estimates of PAR to assess the contribution of PTPN1 to type 2 diabetes in the Caucasian population. PAR estimates (data not shown) range from 17–20%, suggesting that PTPN1 is a significant contributor to type 2 diabetes in Caucasian Americans. A number of assumptions must be made to perform these calculations, however. The two case populations may not be representative. Type 2 diabetic ESRD case subjects are randomly ascertained, but it could be speculated that they are a severely affected type 2 diabetic subgroup based on their ESRD status, and the DHS case subjects are from families with more than one case of type 2 diabetes. Importantly though, the magnitude, e.g., 20% as opposed to 2%, is likely to be reasonably accurate.

Another question that arises is the magnitude of the PTPN1 contribution to the linkage signals that have been observed on chromosome 20. This is not a simple analytical problem for qualitative traits, and our family resources are limited to carry out this test (1). We do, however, have evidence that other genes in the 20q12–13 region may contribute to type 2 diabetes (J.L.B., unpublished observations), suggesting that PTPN1 is not the only diabetes gene in 20q12–13.

Palmer et al. (21) evaluated the impact of PTPN1 polymorphisms on measures of glucose homeostasis in Hispanics, and the results are completely consistent with this report, where PTPN1 polymorphisms and haplotypes are associated with  $S_i$ , a measure of insulin sensitivity, and fasting glucose. This systematic approach in several populations, investigating both diabetes and metabolic traits, suggests that PTPN1 is a significant contributor to diabetes in our population.

## RESEARCH DESIGN AND METHODS

Two independently recruited groups of type 2 diabetic patients and control subjects have been evaluated in this study. The first group is a collection of 300 unrelated Caucasian type 2 diabetic patients with ESRD, with a corresponding collection of 310 randomly ascertained unrelated Caucasian subjects without known diabetes. Both case and control subjects were recruited

simultaneously. This group will be referred to as "type 2 diabetes–ESRD"; their ascertainment and recruitment have been previously described in detail (25). The type 2 diabetes–ESRD subjects have a mean age at diagnosis of diabetes of  $46.5 \pm 12.8$  years, a mean BMI at recruitment of  $28.5 \pm 7.0$  kg/m<sup>2</sup>, a mean maximum reported BMI of  $36.1 \pm 8.3$  kg/m<sup>2</sup>, a mean duration of diabetes >15 years, and a mean HbA<sub>1c</sub> of 8.6%.

The second type 2 diabetes–affected study group consists of type 2 diabetes–affected probands from the DHS (26,27). The DHS is a single-center family-based study of atherosclerosis in type 2 diabetes–enriched families. For the purposes of this report, only the type 2 diabetes proband from each family was defined as a case. A total of 275 type 2 diabetes–affected subjects were genotyped. These subjects (DHS cases) had a mean age of diagnosis of diabetes of  $50.9 \pm 9.9$  years, a mean BMI at recruitment of  $33.7 \pm 7.7$  kg/m<sup>2</sup>, a mean duration of diabetes of 10.9 years, and a mean HbA<sub>1c</sub> of 8.5%; 76% are treated with oral hypoglycemic agents and 33% with insulin. In contrast to the type 2 diabetes–ESRD subjects, DHS subjects were recruited with an exclusion of diabetic nephropathy; DHS cases were not recruited with a complementary control population. As a control population for this case group we used DNA from 200 unrelated (apparently healthy) self-declared Caucasian subjects obtained from the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository (Camden, NJ).

SNPs in this study were selected from the dbSNP database, except for 1484insG (17), P387L (18), and T981C (19). SNPs with frequency information were preferentially selected. The majority of the SNPs had minor allele frequencies >0.2.

Genotyping was performed on a Sequenom MassArray genotyping system using methods previously described (28). Discordance between blind duplicate samples included in the genotyping was <0.9%.

**Statistical analysis.** Pearson's test of homogeneity of proportions was applied to analyze allele frequency and genotype distribution differences between diabetic and nondiabetic subjects. Further analysis was also performed on both allele and genotype frequencies of each SNP using the program CLUMP (29). All analyses used 100,000 simulations, with  $P < 0.05$  considered significant. SNPs were tested for Hardy-Weinberg equilibrium, and pairwise linkage disequilibrium statistics  $D'$  and  $r^2$  were calculated using the SNP-Analysis software package (available from <http://www.fhcr.org/labs/kruglyak/downloads>).

HAPLO.SCORE (30) was used to test for association of haplotypes within the case-control populations. HAPLO.SCORE uses posterior population-based frequency weighting of haplogenotypes that have prior consistency with observed heterozygous diplotypes. Its variance estimates are inflated to account for uncertainty in specific haplotype assignment. We performed a global test of association between PTPN1 haplotypes and type 2 diabetes status followed by individual tests of haplotype risk in a collapsed  $2 \times 2$  analysis. The skip.haplo option was set to 0.01. DANDELION (31), another expectation-maximization algorithm-based program for haplotype analyses was used to calculate haplotype frequencies in specific subject groups.  $P < 0.05$  was considered statistically significant.

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