

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

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Protein tyrosine phosphatase-1B negatively regulates leptin and insulin signaling, potentially contributing to hormonal resistance. We selected six tagging single nucleotide polymorphisms (SNPs) representing 18 common variants in the protein tyrosine phosphatase-1B gene (*PTPN1*) and tested their effect on serum leptin, body fat, and measures of insulin sensitivity and the metabolic syndrome in a large sample of normal female Caucasian twins ($n = 2,777$; mean age, 47.4 ± 12.5 years) from the St. Thomas' U.K. Adult Twin Registry. SNP rs718049 was significantly associated with waist circumference ($P = 0.008$) and central fat ($P = 0.035$) and also with Avignon's insulin sensitivity index (SiM) ($P = 0.007$), fasting insulin ($P = 0.004$), fasting glucose ($P = 0.022$), triglyceride ($P = 0.023$), and systolic blood pressure ($P = 0.046$). SNPs rs2282146 and rs1885177 were associated with SiM ($P = 0.049$ and $P = 0.013$, respectively), and 1484insG was associated with triglyceride ($P = 0.029$). A risk haplotype (7.3%) was associated with lower SiM ($P = 0.036$) and a protective haplotype (5.2%) with higher SiM ($P = 0.057$), with mean values in homozygotes differing by >1 SD ($P = 0.003$). The protective haplotype also showed lower triglyceride ($P = 0.045$) and lower systolic blood pressure ($P = 0.006$). Fine mapping analyses predicted significant associations with SiM and fasting insulin for several ungenotyped SNPs. *PTPN1* variants appear to contribute to central fat and metabolic syndrome traits, secondary to their effect on insulin sensitivity. *Diabetes* 54:3296–3304, 2005

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Received for publication 26 May 2005 and accepted in revised form 27 July 2005.

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Additional information for this article can be found in an online appendix at <http://diabetes.diabetesjournals.org>.

DBP, diastolic blood pressure; GEE, generalized estimating equation; MAF, minor allele frequency; PTP-1B, protein tyrosine phosphatase-1B; SBP, systolic blood pressure; SiM, Avignon's insulin sensitivity index based on both fasting and 2-h insulin and glucose values; SNP, single nucleotide polymorphism; TDT, transmission-disequilibrium test; tSNP, tagging SNP.

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Obesity poses one of the most pressing current public health problems and is a major risk factor for type 2 diabetes. Protein tyrosine phosphatase-1B (PTP-1B) negatively regulates the signaling pathways of insulin and leptin, two hormones involved in the central regulation of energy balance (1). Activated insulin receptor and receptor substrate-1 are dephosphorylated by PTP-1B (2), and phosphorylated JAK2 and STAT3 have been identified as its targets in the leptin pathway (3,4). PTP-1B-deficient mice are hypersensitive to insulin (5) and leptin (4,6) and are protected from diet-induced obesity (4–6). Hence, increased activity of PTP-1B potentially contributes to leptin resistance associated with overweight (7) and resistance to insulin, which predisposes to type 2 diabetes. In humans, *PTPN1*, the gene coding for PTP-1B, maps to a region in which a major human quantitative trait locus for obesity and type 2 diabetes has been reported (8–10). Several rare *PTPN1* single nucleotide polymorphisms (SNPs) have been shown to be associated with insulin resistance and diabetes in different populations (11–14). Recently, Palmer et al. (15) mounted an extensive study of 35 SNPs spanning a 161-kb region including the *PTPN1* gene in >800 Hispanic Americans. Twenty SNPs showed significant association with fasting glucose and the insulin sensitivity index S_i . Through its effect on insulin sensitivity, changes in PTP-1B activity may also be reflected in secondary development of the metabolic syndrome, characterized by increases in central obesity, triglycerides, fasting glucose, and systolic blood pressure (SBP) and lower levels of HDL cholesterol (16). In Caucasians, *PTPN1* SNPs have been associated with serum triglyceride levels in obese subjects (14) and normal individuals (15), and a strong association of common risk haplotypes with hypertension has been found in Chinese and Japanese subjects (17). To the best of our knowledge, no human studies have focused on impaired leptin signal transduction as the underlying pathway by specifically investigating the association of *PTPN1* variants with serum leptin and measures of body fat.

Increased availability of SNPs has recently transformed candidate gene association studies. Examination on a gene-wide level is now recommended with all variants within a candidate gene considered jointly (18). This is best achieved through selection of a minimal set of tagging

TABLE 1
General characteristics of subjects

Variable	<i>n</i>	Means ± SD
Age (years)	2,777*	47.4 ± 12.5
Postmenopausal (%)	2,454	47.7
Obesity-related variables		
Leptin (ng/ml)	2,777	16.5 ± 11.9
BMI (kg/m ²)	2,758	24.7 ± 4.4
Weight (kg)	2,759	65.3 ± 11.8
Waist circumference (cm)	2,707	78.3 ± 10.2
Total fat (kg)	2,718	23.4 ± 8.8
Total fat (%)	2,677	35.6 ± 8.0
Central fat (kg)	2,695	1.33 ± 0.73
Central fat (%)	2,695	31.2 ± 11.5
Insulin sensitivity†		
Fasting glucose (mmol/l)	2,319	4.49 ± 0.56
Fasting insulin (pmol/l)	1,992	6.90 ± 5.40
2-h glucose (mmol/l)	759	5.18 ± 1.10
2-h insulin (μU/ml)	759	33.3 ± 23.1
SiM (10 ¹¹ · l · pmol ⁻¹ · mmol ⁻¹)	759	89.5 ± 68.5
Other components of the metabolic syndrome		
Triglyceride‡ (mmol/l)	2,485	1.27 ± 0.80
HDLc‡ (mmol/l)	2,606	1.55 ± 0.39
SBP§ (mmHg)	2,586	120.9 ± 17.6
DBP§ (mmHg)	2,586	76.1 ± 11.1

*Number of subjects (842 monozygotic and 1,935 dizygotic) with leptin data and genotype data on at least one tSNP. †Nonfasting subjects, patients with either type 1 or type 2 diabetes, and patients on any antidiabetic drugs were all excluded. ‡Subjects using lipid-lowering agents were excluded. §Subjects using antihypertensive medications were excluded. HDLc, HDL cholesterol.

SNPs (tSNPs) effectively capturing information of all common variants by taking into account patterns of linkage disequilibrium across the gene (19,20). We therefore used a multistep design to select a set of tSNPs representing all considered common variants in *PTPN1* and tested the effect of these tSNPs, individually and as haplotypes on a range of variables characterizing body composition, insulin sensitivity and the metabolic syndrome in a sample of Caucasian female twins ($n = 2,777$; mean age, 47.4 ± 12.5 years) that is almost three times the size of the Hispanic population studied by Palmer et al. (15) and 50% larger than the combined Chinese and Japanese cohorts of Olivier et al. (17). Where possible, we compared any specific single SNP and haplotype associations in our larger sample with those found in previous studies. Finally, we performed a fine mapping analysis that uses the tSNPs to predict potential associations between the remaining (ungenotyped) SNPs and the outcome variables, in an attempt to locate potential functional sites (19).

RESEARCH DESIGN AND METHODS

The St. Thomas' U.K. Adult Twin Registry (Twins U.K.) comprises unselected, mostly female volunteers ascertained from the general population through national media campaigns in the U.K. (21). Means and ranges of quantitative phenotypes in Twins U.K. are normally distributed and similar to the age-matched general population in the U.K. (22). General characteristics of the subjects are given in Table 1.

Zygosity, body composition, and biochemical analyses. Zygosity was determined by standardized questionnaire and confirmed by DNA fingerprinting. Serum leptin concentration was determined after an overnight fast using a radioimmunoassay (Linco Research, St. Louis, MO). The majority of these twins also had measures of total and central body fat obtained by dual energy X-ray absorptiometry body composition scans (Hologic QDR-2000; Vertec, Waltham, MA) (23). Blood sample collection for determination of fasting serum insulin and glucose was as described by de Lange et al. (24). Fasting

insulin was measured by immunoassay (Abbott Laboratories, Maidenhead, U.K.) and glucose on an Ektachem 700 multichannel analyzer using an enzymatic colorimetric slide assay (Johnson and Johnson Clinical Diagnostic Systems, Amersham, U.K.). A subsample of twins underwent an oral glucose tolerance test for which glucose and insulin levels were measured before and 2 h after a 75-g oral glucose load. Levels of HDL cholesterol and triglycerides were measured using a Cobas Fara machine (Roche Diagnostics) (25). SBP and diastolic blood pressure (DBP) were measured twice using an automated cuff sphygmomanometer (OMRON HEM713C) and averaged over the two readings. Informed consent was obtained from participants before they entered the study. The protocol was approved by the local research ethics committee.

Genotyping for SNP validation and tSNP selection. Nineteen validated SNPs from the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov/SNP>) and one SNP, 1484insG, reported by Di Paola et al. (13) were selected for validation in the Twins U.K. cohort by genotyping eight random unrelated subjects. Among these 20 SNPs, only rs718050 was not polymorphic. The other 19 SNPs were further genotyped in a sample of 94 unrelated subjects for tSNP selection, using PCR and restriction. This sample size is three times that suggested by one permutation study (26), which indicates that genotyping 25–32 unphased individuals is sufficient to select tSNPs. SNP rs3787339 (minor allele frequency [MAF] of 1%) turned out to be uninformative and was excluded from all further analyses. Primers and PCR conditions for SNP validation and tSNP selection are given in online appendix 1 (available at <http://diabetes.diabetesjournals.org>). The rs number and relative position of these 19 SNPs are shown in Fig. 1.

Genotyping in cohort. Six tSNPs (rs6067484, rs1885177, rs2822146, rs718049, rs3787348, and 1484insG) were selected and genotyped in the complete cohort by Pyrosequencing (Biotage, Uppsala, Sweden). Genotyping accuracy was assessed by inclusion of duplicates (pairs of monozygotic twins) in the arrays and negative controls (water blanks) on each plate. Primers and PCR conditions for SNP genotyping in the full cohort by Pyrosequencing are given in online appendix 2.

Analytical approach. The main purposes of our analyses were to select a set of tSNPs representing the common variants in *PTPN1* and to test the effect of these tSNPs, individually and/or as haplotypes, on a range of variables including serum leptin, measures of body fat, insulin sensitivity, and components of the metabolic syndrome.

tSNP selection. We used two approaches to identify an optimal subset of tSNPs. The first one, devised by Stram et al. (20), selects a subset of SNPs as tSNPs by optimizing their prediction of common haplotypes of all SNPs genotyped in the 94 unrelated individuals. It considers a measure of association (R_H^2) between the true and predicted number of copies (0, 1, or 2) of each possible common haplotype carried by a randomly sampled subject, in which the prediction is based on knowledge of the tSNPs. The program *tagsnps* was used with the following parameters: common haplotypes were defined as “the minimal set of haplotypes that covers 90% of existing haplotypes” and sets of tSNPs resolving the common haplotypes were selected at a R_H^2 threshold of 0.85 because the number of selected tSNPs at this threshold was equal to the number of tSNPs selected using the method of Chapman et al. (19), at an R_L^2 threshold of 0.85 (see below). Chapman et al. selected an optimal set of tSNPs in such a way that the allele frequencies of the remaining (non-tSNPs) can be predicted well. A series of regression equations are calculated for which the predictive efficiency is assessed in terms of R_L^2 , which measures the proportion of variance of each remaining SNP “explained” by regression on the tSNP alleles (locus-based scoring). These regression equations can also be used to predict which of the ungenotyped SNPs might also show association with the trait. The package *htSNP2* was used to select a tSNP set that predicts remaining SNPs with a minimum R_L^2 of 0.85, as suggested by Chapman et al. (19). The program *mipop* implemented in the package *genassoc* predicts the association between unmeasured SNPs and the trait in unrelated subjects. We adapted *mipop* for the analysis of related subjects such as twins by replacing the linear regression with a generalized estimating equations (GEEs) procedure, which yields unbiased standard errors and *P* values (27). The main difference between the approaches of Stram et al. (20) and Chapman et al. (19) is that the former is based on prediction of extended haplotypes (in this case, based on 18 SNPs) from the marker haplotypes (in this case, based on 6 tSNPs), whereas the latter is based on prediction of single SNP loci.

Association analysis. Regular association analyses were performed using GEEs. Analyses were done separately for each of the SNPs and followed up by haplotype analyses. For individual SNP association analyses, we first tested the 2-df (degrees of freedom) codominant model. In the presence of a significant association, a dominant and a recessive model were further tested to find the best mode of inheritance. Age and menopausal status were included as covariates in the models. Details of our approach to test the association of statistically inferred haplotypes with continuous traits have been described previously (28). In short, we used haplotype trend regression

TABLE 2
tSNP selection based on haplotype prediction (R_H^2) and single SNP prediction (R_L^2)

Haplotype	rs3787334	rs6020572	rs932420	rs10485614	rs2426157	rs6067484	rs2426159	rs3787345	rs2038526	rs6060608
1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	1	2	2	2	2	2	2
3	2	2	2	2	1	1	2	1	1	1
4	2	2	2	1	1	1	2	2	2	1
5	2	2	2	1	1	1	2	2	1	1
6	2	2	2	1	1	1	2	2	1	1
7	1	1	1	1	1	1	1	1	1	1
8	2	2	2	1	2	2	2	2	2	2
9	2	2	2	1	2	2	2	2	2	2
10	1	1	1	1	1	1	1	1	1	1
11	2	2	2	2	1	1	2	1	1	2
12	2	2	2	1	1	2	2	2	2	2
13	2	1	2	1	2	2	2	2	2	2
14	2	2	2	1	2	2	2	2	2	2
15	2	2	2	1	1	1	2	2	2	1
16	2	2	2	1	1	1	2	2	2	1
R_L^2	0.965	0.928	0.965	0.882	0.970	—	0.965	0.955	0.961	0.927

tSNPs printed in boldface. R_H^2 , measure of association between the true and predicted haplotype dosage based on knowledge of tSNPs (20). R_L^2 , the proportion of variance of each nontagging SNP explained by regression on the tSNPs (19). Cum freq, cumulative frequency; Freq, frequency.

as outlined by Zaykin et al. (29), with the probabilities of haplotype pairs estimated by PHASE 2.0 software (30). Obesity-related variables included leptin, weight, BMI, waist circumference, total fat mass, percent total fat, central fat mass, and percent central fat. Factor analysis was used to combine strongly correlated indexes of obesity into two measures: one for general obesity (serum leptin, BMI, weight, total fat mass, and percent total fat) and one for central obesity (waist circumference, central fat mass, and percent central fat). To reduce the likelihood of identifying false-positive associations, results of single variables characterizing obesity were confirmed by these two combined scores. We used two indexes of insulin sensitivity: fasting insulin and Avignon's insulin sensitivity index (SiM). SiM is calculated based on both fasting and 2-h insulin and glucose data (31) and was calculated according to the following formulae: $SiM = (0.137 \times SIB + SIH2)/2$, where $SIB = 10^8 / (\text{fasting insulin} \times \text{fasting glucose} \times VD)$; $SIH2 = 10^8 / (2\text{-h insulin} \times 2\text{-h glucose} \times VD)$ and $VD = 150 \text{ ml/kg} \times \text{body wt}$. Both fasting insulin ($r = 0.68$)

and SiM ($r = 0.92$) are highly correlated with insulin sensitivity (S_i) in the normal population. SiM is also a good predictor of diabetes, especially in Caucasians (32). For the metabolic syndrome measures, we included five variables: triglycerides, HDL cholesterol, waist circumference, fasting glucose, and SBP, based on the clinical definition of the insulin receptor substrate 9 (metabolic syndrome) as specified in the third report of the Adult Treatment Panel (ATPIII) of high blood cholesterol (16). To control for population stratification, dizygotic twin pairs discordant for genotype were also used in sibling transmission-disequilibrium test (TDT) association analysis to confirm the results of regular association tests as described elsewhere (27). Preliminary analyses were performed using STATA 8 (StataCorp, College Station, TX). Phenotypes significantly ($P < 0.05$) deviating from normal were log transformed to obtain normal distributions before analysis. Hardy-Weinberg equilibrium was tested by a χ^2 test with 1 df in one twin of each pair chosen

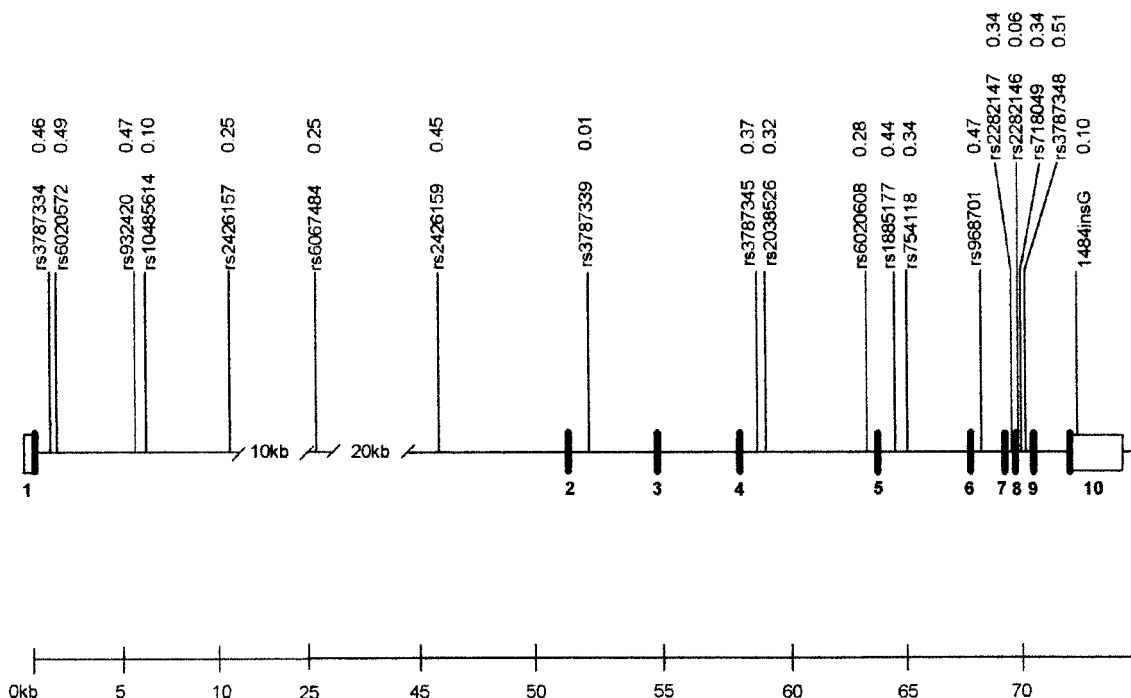


FIG. 1. Genomic map of *PTPN1* gene with locations and MAFs of the validated SNPs genotyped in 94 subjects. ■, exons; □, untranslated regions. MAFs are indicated next to corresponding rs numbers.

TABLE 2
Continued

rs1885177	rs745118	rs968701	rs2282147	rs2282146	rs718049	rs3787348	1484 insG	Freq	Cum freq	R_H^2
1	1	1	1	1	1	2	1	0.471	0.471	0.999
2	2	2	2	1	2	1	1	0.256	0.727	0.899
2	1	2	1	1	1	1	2	0.087	0.814	0.884
2	2	2	2	2	2	1	1	0.042	0.856	0.967
2	1	2	1	1	1	1	1	0.019	0.875	0.990
2	1	2	1	1	2	1	1	0.019	0.894	0.953
1	1	1	1	1	1	1	1	0.019	0.913	0.956
1	2	2	2	1	2	1	1	0.012	0.925	—
2	2	2	2	1	2	1	2	0.012	0.936	—
2	1	1	1	1	1	2	1	0.010	0.946	—
2	1	2	1	1	1	1	2	0.010	0.956	—
2	2	2	2	1	2	1	1	0.010	0.966	—
2	2	2	2	1	2	1	1	0.009	0.975	—
2	2	2	2	2	2	1	1	0.009	0.984	—
1	2	2	2	2	2	1	1	0.008	0.992	—
2	2	2	2	2	2	1	2	0.008	1.0000	—
—	0.961	0.965	0.961	—	—	—	—	—	—	—

at random to prevent inflated significance. Pairwise linkage disequilibrium coefficients were calculated using GOLD and reported as D' and r^2 (33).

RESULTS

Among 20 SNPs tested in the eight subjects, 19 SNPs were polymorphic and genotyped in the 94 subjects. Figure 1 shows the positions and MAFs of these 19 SNPs. All of the SNPs except rs2282146 (P303P in exon 8) were located in noncoding regions, and their genotype frequencies were consistent with Hardy-Weinberg proportions. The uninformative rs3787339 (MAF of 1%) was removed from further analyses (34). The other 18 SNPs had MAFs >5% and showed strong pairwise linkage disequilibrium (online appendixes 3 and 4). The presence of strong linkage disequilibrium throughout the gene suggested the feasibility of tSNP selection, facilitating great savings in time and costs.

Table 2 shows the inferred haplotypes of the 18 SNPs ($n = 94$). Of the seven haplotypes together comprising >90% of the total, three were common (>5%), one had a frequency of 4.2%, and three showed a frequency of 1.9%. The same set of six tSNPs, rs6067484, rs1885177, rs2282146, rs718049, rs3787348, and 1484insG, were selected by *tag-snp*s (20) and *htSNP2* (19) programs. This set of tSNPs accurately predicted both common haplotypes and unmeasured loci. Minimum values of R_H^2 and R_L^2 were 0.884 for haplotype 3 and 0.882 for rs10485614, respectively.

Table 3 shows the genotype and allele frequencies of the six tSNPs in the whole cohort. The total number of subjects genotyped for each polymorphism varied slightly and was somewhat lower than 2,356, the total number of twins genotyped (i.e., one monozygotic and both dizygotic twins of each pair). This was due to unsuccessful amplification of the target sequences for some samples. None of the loci showed deviation from Hardy-Weinberg equilibrium. The inferred haplotype frequencies of the six tSNPs in the whole cohort are also shown in Table 3. The same seven most common haplotypes observed in the 94 subjects (Table 2) were also identified by the six tSNPs, although the actual estimates of haplotype frequencies and corresponding order varied somewhat between the full cohort and the tSNP selection sample ($n = 94$).

Table 4 presents the results of individual SNP analyses. For the SNPs of lowest frequency, rs2282146 and

1484insG, only dominant models were tested, whereas for the four more common SNPs, the 2-df codominant model was first tested, followed by a dominant and a recessive model in the presence of a significant association. With the exception of the effect of rs718049 on waist circumference, none of the main effects of the SNPs on leptin and other obesity-related variables reached statistical significance. We therefore did not include these variables in Table 4. Regular association ($P = 0.074$) and sibling TDT tests ($P = 0.025$) for the combined central obesity score confirmed the significant associations with waist circumference (data not shown). No significant effects of haplotypes on any of the obesity-related variables were found (data not shown). Carriers of the minor allele of rs718049 had significantly higher waist circumference, lower SiM, and higher fasting insulin and glucose. Excepting glucose, these associations were maintained in sibling TDT. SNP rs2282146 was significantly associated with SiM ($P = 0.049$), and this association changed to borderline significance in sibling TDT ($P = 0.062$), with carriers of the minor allele having lower SiM. Under the recessive model, carriers of the minor allele of rs1885177 had significantly lower SiM ($P = 0.013$) and maintained significance in sibling TDT ($P = 0.039$). Two SNPs showed significant associations with other components of the metabolic syndrome. Again, the strongest relationship was shown with rs718049, associated with serum triglyceride and SBP, with all except triglyceride remaining significant in sibling TDT. SNP 1484insG was associated with triglyceride ($P = 0.029$) only in the regular test.

Table 5 shows haplotype frequencies and results of haplotype association tests. Although the overall test did not reach significance ($P = 0.085$), haplotype 4 was significantly associated with lower SiM ($P = 0.036$), i.e., confers risk of insulin resistance, and haplotype 5 was borderline-significantly associated with higher SiM ($P = 0.057$), i.e., is protective. The mean levels of SiM were 0.46 SD lower for haplotype 4 homozygotes and 0.56 SD higher for haplotype 5 homozygotes compared with those homozygous for the most common haplotype 1, a difference of >1 SD ($P = 0.003$). The explained variance of SiM by the *PTPN1* haplotypes was 1.92%. The risk and protective haplotypes are discriminated by rs2282146 and rs718049, reflecting the strongest individual significant associations

TABLE 3
Genotype, allele, and haplotype distributions

tSNP*	Genotype			Total	Hardy-Weinberg test†		MAF (95% CI) (%)
	11	12	22		χ^2	<i>P</i>	
rs6067484	1,093	743	126	1,962	0.18	NS	25.4 (24.0–26.8)
rs1885177	619	1,109	485	2,213	0.03	NS	47.0 (45.5–48.5)
rs2282146	1,713	286	12	2,011	0.22	NS	7.7 (6.9–8.6)
rs718049	813	951	282	2,046	0.01	NS	37.0 (35.5–38.5)
rs3787348	516	998	410	1,924	1.37	NS	47.2 (45.7–48.8)
1484insG	1,697	185	7	1,889	3.82	NS	5.3 (4.6–6.0)
Haplotype‡	rs6067484	rs1885177	rs2282146	rs718049	rs3787348	1484insG	Frequency (SE)
1	1	1	1	1	2	1	0.432 (0.0019)
2	2	2	1	2	1	1	0.242 (0.0016)
3	1	1	1	1	1	1	0.075 (0.0019)
4	1	2	2	2	1	1	0.073 (0.0015)
5	1	2	1	1	1	1	0.052 (0.0024)
6	1	2	1	1	1	2	0.052 (0.0017)
7	1	2	1	2	1	1	0.028 (0.0021)
8	1	1	1	2	2	1	0.016 (0.0010)
9	2	2	1	1	1	1	0.008 (0.0010)

*Includes one monozygotic and both dizygotic twins for each pair. †Tested in one of each monozygotic pair (one of each dizygotic pair and all singleton dizygotic twins). ‡Includes both dizygotic pairs and one of monozygotic pairs with genotype data on at least three tSNPs ($n = 2,320$).

with SiM in the regular and sibling TDT analyses. The protective effects of haplotype 5 were also observed for SBP ($P = 0.006$) and triglyceride ($P = 0.045$) levels. The β -coefficient for haplotype 5 on SBP was -7.2 , and on triglycerides was -0.17 , i.e., SBP levels were 7.2 mmHg lower and triglyceride levels 0.17 mmol/l lower for protective haplotype 5 homozygotes compared with common haplotype 1 homozygotes. The explained percentages of variance in SBP and triglyceride levels by the *PTPN1* haplotypes were 0.33% and 0.63%, respectively.

The approach we used to select an optimal set of tSNPs (19) can also predict which of those SNPs not typed in the full cohort might also show association with the trait. Figure 2 shows the fine mapping results (including the ungenotyped SNPs) based on prediction of single SNP loci from the six tSNPs. Associations of both tSNPs and predicted values for unmeasured SNPs with SiM, fasting insulin, and fasting glucose under the additive genetic model are indicated by $-10\log(P)$. Predicted associations for lipids, SBP, and waist circumference were found to be uninformative and are not shown.

DISCUSSION

Our previous work (21) and previous work by others (35) have shown that variation in leptin levels, total adiposity, and central abdominal fat mass is under strong genetic influence. However, in this study, neither the single SNP analyses nor the haplotype analyses of *PTPN1* showed any significant effect on leptin, weight, BMI, or total fat variables. That is, our results do not support a major role for *PTPN1* variants in body weight regulation, because downstream effects of the hypothesized impairment in leptin signal transduction such as increases in serum leptin and measures of total body fat were not observed. This conclusion is especially important because the design of our study allows us to exclude the most likely alternative explanations for this largely negative result. First, our study did not suffer from a lack of statistical power to detect SNPs with small effects. The current study had 80%

($\alpha = 0.05$) power to detect a biallelic quantitative trait locus explaining as little as 0.5% of the variance (36). Second, strong linkage disequilibrium across *PTPN1* allowed us to effectively capture common variation in this gene by the selected set of tSNPs, making it unlikely that we may have missed any major variants that are either causal or in strong linkage disequilibrium with a causal locus. The third advantage of our study is the comprehensive and accurate measurement of the phenotypes of interest: general and central obesity. In addition to serum leptin levels and anthropometric variables, central and total body fat was assessed by dual energy X-ray absorptiometry, which is a more objective and reliable method of assessing adiposity than, for example, BMI because it allows discrimination between fat, muscle mass, bone, and vital organs (23). Information on all phenotypes was subsequently used to generate one combined score for general obesity and one for central obesity.

Although we observed a small but significant effect of one *PTPN1* variant on central obesity, we suggest this effect is mediated through the inhibition of insulin signaling by PTP-1B, because central fat is a major determinant of insulin sensitivity whereas total fat is the major determinant of leptin levels (23). We found that carriers of the minor alleles of rs1885177, rs2282146, and rs718049 had significantly lower SiM and higher fasting insulin and glucose, associations maintained in sibling TDT. Effects of *PTPN1* on insulin sensitivity and type 2 diabetes risk have been reported by two recent studies (15,37). Palmer et al. (15) found that 20 SNPs with MAFs >0.1 in a single haplotype block covering the *PTPN1* genomic sequence showed significant association with the insulin sensitivity index S_i in Hispanic Americans ($P = 0.003$ – 0.044 , $n = 811$, based on codominant models). Although the study of Palmer et al. is a family study of 55 pedigrees, unlike us, no TDT results were reported to exclude the possibility of false-positive associations due to stratification or admixture. Four of our tSNPs were among those genotyped by Palmer et al.: rs1885177, rs718049, rs3787348, and 1484insG.

TABLE 4
Association analyses of *PTPN1* tSNPs with SiM, fasting insulin, and components of metabolic syndrome

SNP		No.	Means \pm SD			<i>P</i>	
		11/12/22	11	12	22	GEE*	STDT
rs6067484	SiM	342/230/47	89.3 \pm 65.4	91.1 \pm 67.4	88.7 \pm 96.6	NS	NS
	Insulin	921/618/115	6.70 \pm 5.38	6.85 \pm 5.33	7.25 \pm 5.28	NS	NS
	Glucose	1076/745/124	4.48 \pm 0.53	4.50 \pm 0.58	4.41 \pm 0.52	NS	NS
	SBP	1195/808/141	121.1 \pm 17.9	120.3 \pm 17.1	122.1 \pm 17.7	NS	NS
	TG	1171/769/137	1.26 \pm 0.81	1.28 \pm 0.79	1.26 \pm 0.60	NS	NS
	HDLc	1222/800/146	1.58 \pm 0.40	1.52 \pm 0.36	1.53 \pm 0.38	NS	NS
rs1885177	Waist circ	1255/850/150	78.1 \pm 10.2	78.5 \pm 10.1	78.6 \pm 12.4	NS	NS
	SiM	162/302/136	88.6 \pm 67.0	94.9 \pm 75.8	81.6 \pm 70.2	0.013 [†]	0.039 [†]
	Insulin	535/923/415	6.66 \pm 5.19	6.72 \pm 4.89	7.37 \pm 6.43	NS	NS
	Glucose	598/1106/475	4.47 \pm 0.55	4.51 \pm 0.54	4.47 \pm 0.57	NS	NS
	SBP	691/1198/538	121.6 \pm 17.4	120.2 \pm 17.0	121.1 \pm 17.4	NS	NS
	TG	655/1152/518	1.30 \pm 0.81	1.23 \pm 0.75	1.28 \pm 0.89	NS	NS
rs2282146	HDLc	695/1207/542	1.56 \pm 0.39	1.54 \pm 0.38	1.56 \pm 0.39	NS	NS
	Waist circ	708/1270/562	78.1 \pm 10.1	78.4 \pm 9.8	78.6 \pm 10.9	NS	NS
	SiM	553/100/1	90.7 \pm 69.7	81.5 \pm 64.3	19.6 \pm 0.0	0.049 [‡]	0.062 [‡]
	Insulin	1462/238/11	6.93 \pm 5.35	6.81 \pm 5.70	9.86 \pm 6.72	NS	NS
	Glucose	1684/295/11	4.46 \pm 0.55	4.53 \pm 0.57	4.31 \pm 0.46	NS	NS
	SBP	1870/333/14	120.5 \pm 17.6	121.1 \pm 16.9	125.3 \pm 20.9	NS	NS
rs718049	TG	1807/315/13	1.26 \pm 0.77	1.26 \pm 0.92	1.29 \pm 0.87	NS	NS
	HDLc	1894/332/13	1.55 \pm 0.39	1.53 \pm 0.41	1.65 \pm 0.28	NS	NS
	Waist circ	1966/341/12	78.2 \pm 10.1	79.2 \pm 10.9	78.3 \pm 11.0	NS	NS
	SiM	252/300/87	91.6 \pm 68.7	92.1 \pm 71.8	78.4 \pm 78.2	<0.001 [†]	0.002 [†]
	Insulin	681/806/248	6.57 \pm 4.74	6.92 \pm 5.16	7.97 \pm 7.37	0.004 [†]	0.028 [†]
	Glucose	790/937/283	4.47 \pm 0.55	4.51 \pm 0.55	4.54 \pm 0.62	0.045 [†]	NS
rs3787348	SBP	892/1043/315	121.0 \pm 17.6	120.8 \pm 17.4	122.5 \pm 17.4	0.017 [†]	0.025 [†]
	TG	865/986/300	1.25 \pm 0.81	1.29 \pm 0.82	1.28 \pm 0.77	0.023 [‡]	NS
	HDLc	899/1026/318	1.57 \pm 0.41	1.54 \pm 0.38	1.54 \pm 0.38	NS	NS
	Waist circ	933/1094/329	78.0 \pm 9.9	78.6 \pm 9.9	79.1 \pm 11.6	0.022 [‡]	0.008 [‡]
	SiM	173/316/121	92.4 \pm 83.2	92.1 \pm 68.9	87.4 \pm 68.1	NS	NS
	Insulin	426/824/354	7.73 \pm 6.97	6.82 \pm 5.20	6.88 \pm 5.46	NS	NS
1484insG	Glucose	500/961/398	4.52 \pm 0.57	4.49 \pm 0.55	4.49 \pm 0.56	NS	NS
	SBP	582/1078/464	121.2 \pm 18.3	121.0 \pm 17.9	122.3 \pm 17.9	NS	NS
	TG	554/1045/442	1.30 \pm 0.75	1.26 \pm 0.79	1.31 \pm 0.83	NS	NS
	HDLc	578/1101/462	1.53 \pm 0.34	1.55 \pm 0.39	1.54 \pm 0.38	NS	NS
	Waist circ	605/1140/478	78.8 \pm 11.2	78.1 \pm 9.9	77.7 \pm 10.0	NS	NS
	SiM	554/58/3	88.4 \pm 67.5	84.3 \pm 71.6	99.4 \pm 55.2	NS	NS
1484insG	Insulin	1462/148/7	6.94 \pm 5.57	6.67 \pm 4.85	5.39 \pm 2.63	NS	NS
	Glucose	1693/184/7	4.78 \pm 0.56	4.57 \pm 0.57	4.44 \pm 0.36	NS	NS
	SBP	1893/201/6	120.7 \pm 17.2	120.0 \pm 16.9	140.4 \pm 42.0	NS	NS
	TG	1800/204/7	1.28 \pm 0.78	1.15 \pm 0.58	0.96 \pm 0.30	0.029 [‡]	NS
	HDLc	1882/208/7	1.54 \pm 0.38	1.59 \pm 0.40	1.78 \pm 0.47	NS	NS
	Waist circ	1971/212/9	78.3 \pm 10.3	78.2 \pm 10.0	79.7 \pm 8.0	NS	NS

*Age-adjusted *P* value; [†]*P* value under the recessive genetic model; [‡]*P* value under the dominant genetic model. HDLc, HDL cholesterol; TG, triglyceride; Waist circ, waist circumference.

Our results and those of Palmer et al. are consistent in that carriage of the A allele of rs1885177 [rare allele (2) in our cohort and marginally the more frequent allele (1) in that of Palmer et al.] was associated with lower SiM and S_i , as was the allele (2) of rs718049. SNP rs2282146, associated with SiM by us (*P* = 0.049), had a MAF of ~4% in the Hispanic population and did not show a significant influence on S_i . Palmer et al. (15) found that their 20 SNPs were all significantly associated with fasting glucose (*P* = <0.001–0.029), however, we only found an association with rs718049 (*P* = 0.045).

Palmer et al. (15) analyzed eight SNPs tagging haplotypes with frequency >10%, having completed genotyping of all 35 SNPs in their 811 subjects. We reduced effort considerably by first genotyping candidate tSNPs in a small subsample, which confirmed the strong linkage disequilibrium in the region that emerged from the larger

dataset of Palmer et al. and enabled tSNP selection. The eight tSNPs of Palmer et al. included three of our tSNPs, rs718049, rs3787348, and 1484insG, and another three that we had already ascertained were tagged by rs718049: rs3787345, rs745118, and rs2282147. We had not attempted to validate their remaining two SNPs. Palmer et al. (15) defined one risk, one protective, and one neutral haplotype with respect to S_i . We can only directly compare the haplotypes constructed by common SNPs rs718049, rs3787348, and 1484insG in the Hispanic sample and our sample with respect to S_i and SiM. In fact, rs718049 and rs3787348 alone discriminate the Hispanic haplotypes. Haplotype rs718049-rs3787348-1484insG 1-1-1 is protective in the Hispanics and in our twin population. The Hispanic neutral haplotype is also identical to our neutral (most common) haplotype: 1-2-1. The Hispanic risk haplotype 2-2-1 is slightly different; in the twins it is 2-1-1. Therefore,

TABLE 5

Estimated frequencies (>2%) of the six tSNPs haplotypes and association tests with SiM, SBP, and TG

Haplo- type	SNP no.						1484 insG	Frequency	SiM ($10^{11} \cdot 1 \cdot$ $\text{pmol}^{-1} \cdot$ mmol^{-1})		SBP (mmHg)		TG (mmol/l)	
	rs6067484	rs1885177	rs2282146	rs718049	rs3787348	insG			β	<i>P</i>	β	<i>P</i>	β	<i>P</i>
1*	1	1	1	1	2	1	0.4316	—	—	—	—	—	—	
2	2	2	1	2	1	1	0.2417	-6.35	NS	1.16	NS	0.01	NS	
3	1	1	1	1	1	1	0.0751	9.68	NS	-0.51	NS	-0.12	NS	
4	1	2	2	2	1	1	0.0732	-18.6	0.036	1.11	NS	-0.11	NS	
5	1	2	1	1	1	1	0.0521	52.52	0.057	-7.20	0.006	-0.17	0.045	
6	1	2	1	1	1	2	0.0517	-9.63	NS	0.57	NS	-0.14	NS	
7	1	2	1	2	1	1	0.0280	-12.90	NS	1.48	NS	0.38	0.022	
Overall	—	—	—	—	—	—	0.9533	—	0.085	—	0.117	—	0.026	

*Estimates are contrasts with this most common haplotype. TG, triglyceride.

overall, our haplotype results are consistent with those of Palmer et al. with respect to insulin sensitivity. However, unlike them, we found no haplotypes associated with fasting glucose. The same haplotypes associated with S_i and fasting glucose by Palmer et al. (15) have independently been shown to be associated with type 2 diabetes risk and protection in Caucasian type 2 diabetic subjects (37).

Olivier et al. (17) found that of six SNPs in strong linkage disequilibrium spanning *PTPN1*, one SNP (rs16995294, not genotyped by us) was significantly associated with hypertension in 1,553 Chinese and Japanese subjects from 672 families. In a TDT, they identified two 6-SNP haplotypes significantly overtransmitted in hypertensive subjects ($P < 0.0001$). Again, although direct comparisons are not possible, our finding of a protective haplotype for SBP in Caucasians provides further evidence in support of an influence of *PTPN1* on development of hypertension. In the absence of an obvious etiological role, this effect is likely to be secondary to the influence of the gene on insulin sensitivity.

Finally, comparing our study of the 1484insG SNP with that of Di Paola et al. (13) in 477 normoglycemic women from two Italian regions, we found no significant differences in quantitative phenotypes between wild-type and

1484insG carriers, including those for which they found positive associations: SBP and DBP (higher in one group only) and elevated 1-h and 2-h insulin levels (measured in the other group). They found that 1484insG was associated with higher serum triglycerides in Italian men but not in women, however, we found an association in women, which remained significant after adjustment for BMI ($P = 0.029$).

Variation in the coding region of *PTPN1* is relatively rare (only two nonsynonymous changes listed on the National Center for Biotechnology Information database), and associations of the synonymous coding or intronic SNPs studied by us are likely to result from linkage disequilibrium with functional variants, rather than be causal themselves. We can use the prediction rules computed during the choice of the marker subset to target specific loci for further study (19). With regard to SiM and fasting insulin, we predict additional significant associations with rs3787345 and rs2038526 in intron 4, rs754118 in intron 5, and rs2282147 in intron 7. Although it remains difficult to predict where the causal locus or loci might be due to the strong linkage disequilibrium across this gene, Fig. 2 indicates that certain regions of the gene can be excluded and others need to be considered when looking for causal loci and, as such, provides useful direction. The large

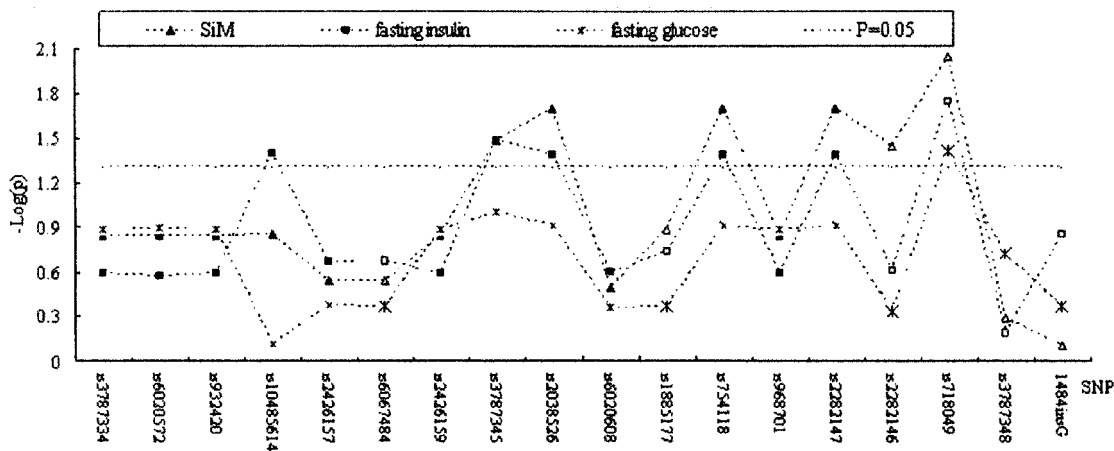


FIG. 2. Associations of *PTPN1* SNPs (both unmeasured SNPs and tSNPs) with SiM, fasting insulin, and fasting glucose under the additive genetic model. Associations of tSNPs and predicted values for unmeasured SNPs are indicated by $-\log(P)$. Points above the dotted line representing $-\log(P) = 0.05$ represent SNP associations found or predicted to be significant at the $P = 0.05$ level.

introns in *PTPN1* and the flanking regions may contain unknown regulatory elements; for example, the 3'-untranslated region 1484insG variant was shown to stabilize PTP1-B mRNA in vitro (13). We are now validating SNPs in the *PTPN1* 5'-flanking region and will explore whether they are in strong linkage disequilibrium with the existing tSNPs (especially rs2282146), which could be the causal locus or loci. Our findings and those of others relating *PTPN1* variation to insulin resistance, lipid profile, central obesity, and hypertension suggest that this gene has a significant influence on progression of the metabolic syndrome. Demonstration of an etiological *PTPN1* variant is eagerly awaited and would confirm PTP1-B as an influential factor.

ACKNOWLEDGMENTS

This study has received funding from British Heart Foundation Project Grant PG/04/028. The Twin Research and Genetic Epidemiology Unit has received support from the Wellcome Trust, Arthritis Research Campaign, the Chronic Disease Research Foundation, and the European Union 5th Framework Programme Genom EU twin no. QL2-CT-2002-01254.

This research was conducted within the network of the London IDEAS Genetic Knowledge Park, and used the St. George's, University of London Medical Biomics Centre.

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