Protein tyrosine phosphatase 1B is not a major susceptibility gene for type 2 diabetes mellitus or obesity among Pima Indians

M. Traurig · R. L. Hanson · S. Kobes · C. Bogardus ·
L. J. Baier

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Abstract

Aim/hypothesis Single-nucleotide polymorphisms (SNPs) in the protein tyrosine phosphatase 1B gene (*PTPNI*) have been reported to be associated with type 2 diabetes in white subjects, and insulin sensitivity and fasting glucose levels in Hispanic Americans. In this study, we determined whether SNPs in *PTPNI* also have a role in type 2 diabetes susceptibility in Pima Indians, a population with the world's highest reported prevalence and incidence rates of this disease.

Materials and methods Thirty-one SNPs across a 161-kb region encompassing *PTPNI* were genotyped in 1,037 Pima Indians for association studies with type 2 diabetes and obesity.

Results Twenty-five of the SNPs had allele frequencies >0.05, and these SNPs fell into two linkage disequilibrium blocks ($D' > 0.9$). Block 1 contains six SNPs that span a 61-kb region upstream of *PTPNI*, while block 2 contains 19 SNPs that cover the entire *PTPNI* gene. None of the SNPs, analysed individually or as haplotypes, was associated with either type 2 diabetes or obesity. However, three SNPs located

in block 1 were nominally associated (p values ranging from 0.01 to 0.05) with insulin sensitivity as measured by the hyperinsulinaemic–euglycaemic clamp technique.

Conclusions/interpretation Based on our association results, we conclude that SNPs within *PTPNI* are unlikely to have a major role in the aetiology of type 2 diabetes or obesity in Pima Indians.

Keywords Genotyping · Insulin sensitivity · Obesity · Pima Indians · *PTPNI* · SNPs · Type 2 diabetes

Abbreviations

LD	linkage disequilibrium
<i>PTPNI</i>	protein tyrosine phosphatase 1B gene
SNP	single-nucleotide polymorphism
tSNP	tagging SNP

Introduction

The gene for protein tyrosine phosphatase 1B (*PTPNI*) has previously been investigated as a positional candidate gene for the linkage to type 2 diabetes reported on chromosome 20q13 [1]. In a study of white subjects, Bento et al. observed associations between several non-coding single-nucleotide polymorphisms (SNPs) positioned across *PTPNI* and type 2 diabetes [2], whereas, in a study of Hispanic American subjects, Palmer et al. observed associations between several of the same SNPs and insulin sensitivity and/or fasting glucose levels [3]. Subsequent replication studies of these initial reports have provided weaker or negative results for associations with type 2 diabetes or related phenotypes [4–6]. In the current study, we sought to determine whether SNPs in *PTPNI* have a

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M. Traurig · R. L. Hanson · S. Kobes · C. Bogardus · L. J. Baier
Diabetes Molecular Genetics Section, Phoenix Epidemiology
and Clinical Research Branch, National Institute of Diabetes,
Digestive and Kidney Diseases, National Institutes of Health,
Phoenix, AZ, USA

L. J. Baier (✉)
National Institute of Diabetes, Digestive and Kidney Diseases,
National Institutes of Health,
445 North 5th Street,
Phoenix, AZ 85004, USA
e-mail: lbaier@phx.niddk.nih.gov

role in susceptibility to type 2 diabetes or obesity in the Pima Indian population of Arizona.

Subjects and methods

Subjects Subjects are part of our ongoing longitudinal study of type 2 diabetes among the Gila River Indian Community. All subjects provided written informed consent prior to participation. This study was approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National Institute of Diabetes, Digestive and Kidney Diseases. DNA from 1,037 Pima Indian subjects from 332 nuclear families was used for genotyping. Diabetes status was determined using a 75 g OGTT interpreted by the criteria of the WHO. For the diabetic subjects ($n=573$), 64.4% were female, BMI=34.4±8.5 kg/m², mean age 44.7±13 years, and mean age at diagnosis 33.9±10.8 years. For the non-diabetic subjects ($n=464$), 43.5% were female, mean BMI=33.5±8.4 kg/m², mean age 31.4±10.6 years. In this population the cumulative incidence of type 2 diabetes by age 31 is ~20% [7].

Genotyping of SNPs Thirty-one previously analysed SNPs near or within *PTPN1* [2–4, 6] were genotyped by SNPlex (Applied Biosystems, Foster City, CA, USA) following the manufacture's protocol or by allelic discrimination PCR (Assays-by-Design Service; Applied Biosystems). Genotyping of the SNPs included 100 blind duplicate samples. All duplicated samples had a concordance of >98%. The genotype success rate for the 1,037 samples and 100 blind duplicates was 98.6% (range 97.6–100%).

Statistical analysis Statistical analyses were performed using the statistical analysis system of the SAS Institute (Cary, NC, USA). For continuous variables, the general estimating equation procedure (GEE) was used to adjust for appropriate covariates (see footnotes to Tables 1 and 2). The GEE method accounts for familial relationships (sibship) by modelling the correlation among family members. A p value=0.05 was considered statistically significant. Linkage disequilibrium (LD) between SNPs was calculated using the Estimating Haplotypes (EH) program. For pairs of SNPs, D' was calculated as a measure of allelic association and r^2 as a measure of concordance. Haplotype frequencies were calculated using the EH program, and associations between traits and individual haplotypes were examined with a modification of the zero-recombinant haplotyping procedure [8].

Clinical tests Body composition was estimated by underwater weighing or dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation Corp., Madison, WI, USA), and a conversion equation was used to make the estimates comparable

between the two methods. Oral glucose tolerance was assessed following 2–3 days on a weight-maintaining diet. Blood was drawn prior to ingestion of 75 g glucose and at 30, 60, 120 and 180 min thereafter. On another day, subjects received a 25 g i.v. injection of glucose over 3 min to measure the acute insulin response. Blood samples were collected prior to infusion and at 3, 4, 5, 6, 9 and 10 min thereafter. The acute insulin response was calculated as half the mean increment in plasma insulin concentrations from 3 to 5 min.

For the hyperinsulinaemic–euglycaemic clamp studies, both basal glucose appearance and insulin-stimulated glucose disappearance (uptake) rates were determined as described elsewhere [9]. Briefly, insulin was infused to achieve physiological plasma insulin concentrations (952 ± 21 pmol/l) for 100 min. Plasma glucose concentrations were held at ~5.6 mmol/l by variable 20% glucose infusion. Tritiated glucose was infused for 2 h before the insulin infusion to calculate both post-absorptive glucose appearance rates and glucose disappearance rates during the insulin infusion. Glucose appearance and disappearance rates were normalised to estimated metabolic size (fat-free mass+17.7) [9].

Results and discussion

Thirty-one previously reported SNPs near or within *PTPN1* were genotyped in 1037 Pima Indians. None of the SNPs deviated from Hardy–Weinberg equilibrium. Five of the SNPs were monomorphic in the Pima population. Twenty-five of the remaining 26 SNPs fell into two haplotype ‘blocks,’ defined by contiguous SNPs with $D' > 0.9$ (Electronic supplementary material [ESM] Fig. 1). Six SNPs upstream of *PTPN1* define block 1 and 19 SNPs that span the *PTPN1* gene define block 2 (ESM Fig. 1). Due to its low frequency (<0.05), rs3787335 was not considered in the LD analysis. None of the 26 polymorphic SNPs was associated with either type 2 diabetes or BMI (Table 1). Although the sample size of the 1,037 subjects is comparable or larger than the Bento et al. [2] and Palmer et al. [3] studies, it may nonetheless have low statistical power to detect variants with modest effects. The power to detect an association depends on the number of case and control subjects, the prevalence of disease and the effect of the associated alleles (e.g. the locus-specific heritability). Given the current sample size and prevalence of 55%, we estimate that the power of our study is >70% to detect a significant ($p<0.05$) association with a causal variant with minor allele frequency >0.05 accounting for 1% of the variance in liability to diabetes [10].

The 26 SNPs were also analysed for associations with predictors of type 2 diabetes and obesity in a subgroup of 264 non-diabetic full-heritage Pima Indians who had

Table 1 Association analyses of the 31 *PTPN1* SNPs with type 2 diabetes mellitus and BMI

SNP	Prior studies	Alleles M/m	mAF	Diabetic			Non-diabetic			<i>p</i> value ^a	OR (95% CI) ^b	Mean BMI (kg/m ²)			
				M/M (%)	M/m (%)	m/m (%)	M/M (%)	M/m (%)	m/m (%)			M/M	M/m	m/m	<i>p</i> value ^a
rs2904268		G/G	Mono	—	—	—	—	—	—	—	—	—	—	—	—
rs803742		C/T	0.21	348 (62)	192 (34)	18 (3)	281 (62)	151 (33)	21 (5)	0.65	1.07 (0.81–1.41)	35.8	35.8	34.9	0.95
rs1967439		A/G	0.38	206 (38)	262 (48)	81 (15)	168 (38)	217 (48)	63 (14)	0.94	1.01 (0.80–1.28)	35.6	36.0	35.5	0.61
rs6020546	^c	C/T	0.06	506 (91)	52 (9)	0 (0)	387 (86)	61 (14)	0 (0)	0.17	0.99 (0.78–1.25)	35.9	34.3	—	0.16
rs4811074		C/T	0.38	214 (38)	263 (47)	81 (15)	172 (38)	221 (49)	63 (14)	0.98	1.00 (0.79–1.27)	35.6	35.9	35.4	0.50
rs4811075		G/A	0.38	210 (38)	257 (47)	81 (15)	169 (38)	217 (49)	60 (14)	0.82	1.03 (0.82–1.28)	35.4	35.8	35.5	0.71
rs718630	^{d,f}	T/G	0.36	70 (13)	251 (46)	227 (41)	54 (12)	208 (47)	182 (41)	0.95	0.97 (0.78–1.22)	35.3	35.9	35.6	0.30
rs4811078	^d	C/C	Mono	—	—	—	—	—	—	—	—	—	—	—	—
rs6512651		C/G	0.38	219 (40)	256 (46)	79 (14)	162 (36)	223 (50)	66 (15)	0.36	1.11 (0.88–1.40)	35.9	35.6	35.0	0.33
rs3787334	^{e,f}	T/C	0.41	219 (39)	261 (47)	81 (14)	163 (36)	224 (50)	62 (14)	0.67	1.05 (0.83–1.33)	36.0	35.7	34.9	0.16
rs2206656	^{d,e,f}	C/G	0.38	220 (40)	255 (46)	79 (14)	161 (37)	216 (49)	63 (14)	0.46	1.09 (0.87–1.38)	36.0	35.8	35.0	0.29
rs93240	^{e,f}	C/T	0.39	83 (15)	259 (46)	219 (39)	68 (15)	224 (49)	161 (36)	0.44	1.09 (0.87–1.38)	35.0	35.8	36.0	0.19
rs2426156		T/G	0.38	222 (40)	256 (46)	76 (14)	162 (36)	221 (49)	65 (15)	0.31	1.13 (0.89–1.43)	36.0	35.9	35.0	0.27
rs3787335		G/T	0.03	0 (0)	35 (7)	520 (94)	0 (0)	24 (5)	429 (95)	0.80	0.92 (0.48–1.76)	—	35.1	35.7	0.47
rs2426158	^{e,f}	A/G	0.39	218 (39)	263 (47)	79 (14)	160 (35)	232 (51)	66 (14)	0.31	1.13 (0.89–1.42)	36.0	35.7	35.1	0.24
rs2904269	^{e,f}	C/A	0.41	196 (35)	271 (49)	88 (16)	149 (33)	231 (51)	74 (16)	0.40	1.11 (0.88–1.39)	36.2	35.4	35.1	0.11
rs1570179	^{e,f}	C/T	0.41	196 (35)	266 (48)	82 (15)	144 (33)	227 (52)	70 (16)	0.27	1.15 (0.90–1.46)	36.1	35.4	35.0	0.09
rs3787345	^{d,e,f}	C/T	0.41	81 (16)	254 (49)	184 (36)	62 (15)	223 (53)	135 (32)	0.44	1.10 (0.86–1.42)	35.5	35.3	36.0	0.27
rs2038526	^{e,f}	C/T	0.40	201 (38)	246 (46)	85 (16)	150 (35)	216 (50)	67 (16)	0.48	1.09 (0.86–1.39)	36.2	35.4	35.4	0.29
rs1885177	^{e,f,g}	A/C	0.42	89 (16)	271 (49)	194 (35)	73 (16)	232 (52)	145 (32)	0.39	1.11 (0.88–1.41)	35.4	35.5	36.2	0.17
rs754118	^{d,e,f}	C/T	0.41	199 (36)	270 (49)	87 (16)	147 (33)	230 (51)	73 (16)	0.34	1.12 (0.89–1.41)	36.2	35.4	35.5	0.23
rs3215684	^{e,f}	O/T	0.41	193 (35)	265 (49)	87 (16)	145 (33)	223 (51)	68 (16)	0.54	1.08 (0.85–1.36)	36.0	35.5	35.3	0.18
rs968701	^{e,f}	A/G	0.41	198 (36)	267 (49)	85 (16)	149 (33)	229 (51)	70 (16)	0.43	1.10 (0.87–1.39)	36.3	35.4	35.4	0.19
rs2282147	^{d,e,f}	A/G	0.41	86 (16)	276 (50)	193 (35)	70 (16)	231 (52)	144 (32)	0.45	1.10 (0.86–1.39)	35.5	35.5	36.1	0.23
rs718050	^{d,e,f}	A/G	0.40	80 (15)	260 (49)	187 (36)	60 (14)	220 (52)	147 (34)	0.94	1.01 (0.79–1.29)	35.6	35.4	36.0	0.30
rs3787348	^{d,e,f}	G/T	0.42	90 (16)	272 (49)	196 (35)	75 (17)	231 (51)	147 (33)	0.37	1.11 (0.88–1.41)	35.4	35.5	36.2	0.16
G381S		G/G	Mono	—	—	—	—	—	—	—	—	—	—	—	—
P387L	^{c,h,i}	C/C	Mono	—	—	—	—	—	—	—	—	—	—	—	—

Table 1 (continued)

SNP	Prior studies	Alleles M/m	mAF	Diabetic			Non-diabetic			<i>p</i> value ^a	OR (95% CI) ^b	Mean BMI (kg/m ²)			
				M/M (%)	M/m (%)	m/m (%)	M/M (%)	M/m (%)	m/m (%)			M/M	M/m	m/m	<i>p</i> value ^a
rs2426164	e,f	A/G	0.41	198 (36)	272 (49)	86 (16)	150 (33)	235 (52)	71 (16)	0.36	1.12 (0.88–1.41)	36.2	35.4	35.1	0.10
1484insG	d,e,f	G/O	Mono	—	—	—	—	—	—	—	—	—	—	—	—
rs1060402	f	A/G	0.41	195 (35)	268 (49)	88 (16)	149 (33)	225 (50)	74 (17)	0.44	1.10 (0.87–1.38)	36.0	35.5	34.7	0.07

Data are *n* (%)

M, major allele; m, minor allele; mAF, minor allele frequency

^a*p* values were calculated under the additive model and adjusted for age, sex, birth date, family membership and Pima heritage

^bAll odds ratios (ORs) and 95% CIs were calculated per copy of the major allele by logistic regression and adjusted for age, sex, birth date, family membership and Pima heritage

^cSNPs associated with type 2 diabetes in white subjects [4]

^dSNPs associated with type 2 diabetes in white subjects [2]

^eSNPs associated with insulin sensitivity in Hispanic Americans [3]

^fSNPs associated with fasting glucose levels in Hispanic Americans [3]

^gSNPs associated with insulin sensitivity in female white twins [5]

^hSNPs associated with fasting insulin levels and glucose disappearance in white subjects from the HERITAGE Family study [11]

ⁱSNPs associated with type 2 diabetes in the Danish population [12]

undergone detailed metabolic testing including measurements of body composition, oral glucose tolerance, insulin secretory function and insulin action. Three SNPs in block 1 (rs196749, rs4811074 and rs4811075) were nominally associated with insulin-stimulated glucose uptake (insulin sensitivity) during a hyperinsulinaemic–euglycaemic clamp (Table 2; rs4811074 is shown as a representative SNP). In the study by Palmer et al. [3] several SNPs were associated with insulin sensitivity [3] (Table 1), but these are different

SNPs positioned within block 2. Consistent with the Palmer study, Spencer-Jones et al. also reported associations between SNPs within block 2 and measures of insulin sensitivity [5] (Table 1). Therefore, it is possible that the nominal associations observed between SNPs in block 1 and insulin sensitivity in Pima Indians represent false associations due to multiple testing, rather than independent confirmation that this gene has a role in insulin action. Palmer et al. also detected two SNPs (rs4811077 and

Table 2 Clinical characteristics of the 264 non-diabetic Pima Indians by genotype for SNP rs4811074

	Means			<i>p</i> value
	M/M (95% CI)	M/m (95% CI)	m/m (95% CI)	
<i>n</i> (female/male)	31/70	59/72	15/17	
BMI (kg/m ²) ^a	34 (33–35)	34 (33–35)	34 (32–37)	0.56
Body fat (%) ^a	32 (31–34)	33 (31–35)	33 (30–36)	0.27
Fasting plasma glucose (mmol/l) ^b	5.00 (4.89–5.11)	5.11 (5.00–5.17)	5.17 (4.94–5.33)	0.23
2 h glucose (mmol/l) ^b	7.00 (6.67–7.33)	7.00 (6.72–7.33)	7.22 (6.61–7.78)	0.47
Fasting plasma insulin (pmol/l) ^b	250 (236–271)	243 (222–264)	285 (236–340)	0.31
30-min insulin (pmol/l) ^c	1,368 (1,188–1,584)	1,542 (1,375–1,736)	1,743 (1,347–2,257)	0.17
2 h insulin (pmol/l) ^b	1,035 (889–1,215)	1,160 (1,007–1,340)	1,222 (917–1,625)	0.18
Glucose uptake rate (mg kg EMBS ⁻¹ min ⁻¹) ^b	3.50 (3.31–3.69)	3.46 (3.31–3.61)	3.24 (3.02–3.48)	0.03
Acute insulin response (pmol/l) ^d	1,389 (1,202–1,597)	1,549 (1,375–1,764)	1,715 (1,264–2,334)	0.11

Only non-diabetic subjects were used in this analysis

p values were calculated under the additive model and were adjusted for:

^aage, sex and family membership

^bage, sex, per cent body fat and family membership

^cage, sex, per cent body fat, glucose disposal rate, 30-min glucose levels and family membership; analysis for 30-min insulin was done only in normal glucose tolerant subjects, *n*=186

^dage, sex, per cent body fat, glucose disposal rate and family membership; analysis for acute insulin response was done only in normal glucose tolerant subjects, *n* (female/male): 1/1, 74 (17/57); 1/2, 91 (36/55); 2/2, 21 (8/13)

EMBS, estimated metabolic body size

1484insG) that were associated with the acute insulin response (AIR) [3], while Ukkola et al. detected a single SNP (rs968701) that was associated with the AIR in white subjects [11]. None of the SNPs was associated with an AIR among 186 full-heritage Pima Indians with normal glucose tolerance (data shown for rs4811074 in Table 2).

In their study of white subjects, Bento et al. also identified a common risk haplotype (frequency 36%) consisting of eight SNPs that was associated with type 2 diabetes [2]. This risk haplotype was also associated with increased insulin resistance and higher fasting glucose levels in Hispanics [3]. In Pima Indians, seven of these eight SNPs had $r^2 > 0.9$ and the eighth SNP (1484insG) was monomorphic; therefore, this haplotype could be defined by a single SNP (e.g. the C allele at rs3787345) in Pima Indians where there was no association with type 2 diabetes. However, we further examined the Pima-specific haplotypes within the two LD blocks for association with type 2 diabetes. Among Pima subjects, there was a high redundancy ($r^2 > 0.9$) between SNPs in each block; thus, tagging SNPs (tSNPs) were selected to define common haplotypes. Three tSNPs (rs803742, rs6020546 and rs718630) define the four haplotypes for block 1, while two tSNPs (rs2426158 and rs1060402) define the two haplotypes for block 2. No associations were found between any of the six haplotypes and type 2 diabetes (ESM Table 1). This is consistent with the Florez et al. study that examined five haplotypes in the *PTPN1* region in Genomics Collaborative Inc. (GCI) US, GCI Poland and Scandinavian populations but did not observe any associations between the haplotypes and type 2 diabetes [4]. Florez et al. further examined whether the two haplotypes (risk and protective) observed in the Bento et al. [3] and Palmer et al. [4] studies were associated with fasting plasma glucose levels and insulin sensitivity in the GCI US and Scandinavian populations. The previously observed risk haplotype was associated with higher fasting plasma glucose levels in the GCI US population, but not in the Scandinavian population, or the combined populations [4].

In conclusion, our association analyses of SNPs and haplotypes near/within the *PTPN1* locus suggest that this gene does not have a major role in either type 2 diabetes or obesity among the Pima Indians. However, since three SNPs upstream of *PTPN1* were nominally associated with a measure of insulin sensitivity in non-diabetic Pima Indians, we cannot exclude the possibility that *PTPN1*, or a nearby gene, has some minor role in influencing insulin action in this Native American population.

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Duality of interest The authors confirm that there is no duality of interest.

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