

A P387L Variant in Protein Tyrosine Phosphatase-1B (PTP-1B) Is Associated With Type 2 Diabetes and Impaired Serine Phosphorylation of PTP-1B In Vitro

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In the present study, we tested the hypothesis that variability in the protein tyrosine phosphatase-1B (PTP-1B) gene is associated with type 2 diabetes. Using single-strand conformational polymorphism analysis, we examined cDNA of PTP-1B from 56 insulin-resistant patients with type 2 diabetes as well as cDNA from 56 obese patients. Four silent variants, (NT CGA→CGG) R199R, (NT CCC→CCT) P303P, 3'UTR+104insG, and 3'UTR+86T→G, and one missense variant, P387L, were found. Subsequent analysis on genomic DNA revealed two intron variants, IVS9+57C→T and IVS9+58G→A, and two missense variants, G381S and T420M. The G381S and 3'UTR+104insG insertion variants were not associated with type 2 diabetes. In an association study, the P387L variant was found in 14 of 527 type 2 diabetic subjects (allelic frequency 1.4%, 0.4–2.4 CI) and in 5 of 542 glucose-tolerant control subjects (allelic frequency 0.5%, CI 0.1–1.1), showing a significant association to type 2 diabetes ($P = 0.036$). In vitro, p34 cell division cycle (p34^{cdc2}) kinase-directed incorporation of [γ -³²P]ATP was reduced in a mutant peptide compared with native peptide (387P: 100% vs. 387L: $28.4 \pm 5.8\%$; $P = 0.0012$). In summary, a rare P387L variant of the PTP-1B gene is associated with a 3.7 (CI 1.26–10.93, $P = 0.02$) genotype relative risk of type 2 diabetes in the examined population of Danish Caucasian subjects and results in impaired in vitro serine phosphorylation of the PTP-1B peptide. *Diabetes* 51:1–6, 2002

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IRS, insulin receptor substrate; p34^{cdc2}, p34 cell division cycle; MBP, myelin basic protein; PCR, polymerase chain reaction; PTP-1B, protein tyrosine phosphatase-1B; SSCP, single-strand conformation polymorphism.

Type 2 diabetes is a complex disorder with a strong genetic background (1). The multiple clinical manifestations of type 2 diabetes appear to originate from both defective insulin secretion as well as from an impaired action of insulin in target tissues (2). After binding of insulin, signaling through the insulin receptor is mediated by tyrosine phosphorylation of downstream signaling proteins such as insulin receptor substrate (IRS)-1 and -2, which initiate a signaling cascade (3). Modulators of this cascade include protein tyrosine phosphatases that regulate the activity of multiple cellular proteins by controlling their phosphorylation status (4). The protein tyrosine phosphatase-1B (PTP-1B) is an ubiquitously expressed tyrosine phosphatase; in in vitro studies, PTP-1B blocks the insulin-stimulated tyrosine phosphorylation of the insulin receptor and thereby the IRS-1, two important mediators of insulin signaling (5,6). Intriguingly, PTP-1B localizes to the endoplasmic reticulum through a 35-aa COOH-terminal motif, extending the phosphatase domain toward the cytoplasm (7). It has recently been shown that mice deficient in PTP-1B exhibit increased insulin sensitivity as well as resistance to weight gain when fed a high-fat diet (8,9). The increased insulin sensitivity correlated to increased insulin-stimulated phosphorylation of the insulin receptor and IRS-1 (8). Also, recent reports indicate that upregulation of PTP-1B may be involved in the pathogenesis of both obesity and type 2 diabetes by blocking the insulin cascade signal transduction in vivo (10,11). Given the key role of PTP-1B, we hypothesized that variations in the human PTP-1B gene modulate insulin sensitivity and/or insulin secretion and predispose to type 2 diabetes. Because the genomic characterization of the human PTP-1B was only recently published (12), we analyzed the coding region of the PTP-1B gene for mutations potentially involved in the pathogenesis of type 2 diabetes in muscle cDNA from 56 insulin-resistant type 2 diabetic subjects and adipose tissue cDNA from 56 obese subjects.

RESEARCH DESIGN AND METHODS

Study groups. The primary mutation analysis was performed on cDNA derived from muscle biopsies from 56 unrelated insulin-resistant type 2 diabetic patients as previously described (13) and on cDNA derived from

TABLE 1

Nucleotide sequences of primers used for PCR amplification of the PTP-1B cDNA segments for SSCP-heteroduplex gel analyses and sequencing of variants

Segment	Primers (nt nr) 5' → 3' F: Forward R: Reverse	Size (bp)	Annealing temperature (°C)	Concentration MgCl ₂ (mmol/l)
Primary (1°)	PF: (7)-GCCTCGGGGCTAAGAGC PR: (1645)-AGAGAGTACCATGCTGGCG	1,657	58	2.0
1	1F: (49)-CAGTGGGCCGAGAAGGA 1R: (292)-AACGCTAGTTTGATAAAAAATGGAA	267	58	1.5
2	2F: (258)-GATTAAGTACATCAAGAAGA 2R: (525)-CTCTGAAGATATCAAGTCATA	288	54	1.5
3	3F: (484)-GAGATGATCTTTGAAGACAC 3R: (759)-AACCTTCTGTCTGGCTGATA	295	54	1.5
4	4F: (677)-TCAAAGTCCGAGAGTCAGG 4R: (941)-ACTCTTCCGTGCAGGATCA	283	57	1.5
5	5F: (900)-CTACCTGGCTGTGATCGAAG 5R: (1189)-GACACTGAAGTTAGAAGTCGGG	311	59	1.5
6	6F: (1152)-AAATGCCGCACCCTACG 6R: (1447)-AGAGCCCACGCCCGACTA	313	58	1.5
7	7F: (1368)-AAATGCCGCACCCTACG 7R: (1645)-AGAGAGTACCATGCTGGCG	296	60	2.0

Nucleotide numbers (according to accession number M31724) of the first nucleotide (5') in each primer are given in parentheses.

subcutaneous fat biopsies from 56 unrelated obese patients (age 42.5 ± 11.7 years and BMI 43.2 ± 6.1 kg/m²). The association studies of the G381S, P387L, and 3'UTR+104insG variants were performed using 527 unrelated type 2 diabetic patients (58% men and 42% women), who were recruited from the outpatient clinic at Steno Diabetes Center. The mean age of the patients was 60.0 ± 11.0 years, and their mean BMI was 29.0 ± 5.0 kg/m². Twenty-eight percent of the patients were treated with diet, 57% with an oral hypoglycemic agent, and 15% with insulin. The association studies also comprised 542 unrelated age-matched glucose-tolerant control subjects (49% men and 51% women) who were traced in the central population register and living in the same area of Copenhagen as the type 2 diabetic patients examined at Steno Diabetes Center ($n = 240$) (14) and in the Copenhagen County Center of Preventive Medicine during 1994–1997 ($n = 302$) (15). The control subjects had a mean age of 56 ± 10 years and a mean BMI of 25 ± 8 kg/m². All participants underwent a 75-g oral glucose tolerance test. Diabetes was diagnosed in accordance to the 1985 World Health Organization criteria (16). Only normal glucose-tolerant subjects were used in the association study, and all study participants were Danish Caucasians by self-report. The studies were approved by the Ethics Committee of Copenhagen (KA-96008) and were carried out in accordance with Helsinki Declaration II. Before participating in the study, informed consent was obtained from all subjects.

Biochemical assays. Blood samples for measurement of serum levels of insulin, total cholesterol, HDL cholesterol, triglycerides, and plasma glucose were drawn after a 12-h overnight fast. Serum triglycerides, total serum cholesterol, serum HDL cholesterol, and serum LDL cholesterol were analyzed using enzymatic colorimetric methods (GPO-PAP and CHOD-PAP; Boehringer Mannheim, Mannheim, Germany). The plasma glucose concentration was analyzed by a glucose oxidase method (Granutest; Merck, Darmstadt, Germany); serum-specific insulin, excluding des(31,32) and intact proinsulin, was measured by enzyme-linked immunosorbent assay (Dako insulin kit K6219; Dako Diagnostics, Ely, U.K.). HbA_{1c} was measured by ion-exchange high-performance liquid chromatography (nondiabetic reference range: 4.1–6.4%).

Mutation analyses. We conducted a primary mutation analysis on cDNA prepared from total RNA extracted from 56 biopsies from the vastus lateralis muscle and 56 subcutaneous fat biopsies according to standard procedures as previously described (13,17). The PTP-1B cDNA was amplified by a primary polymerase chain reaction (PCR) segment covering parts of the 5' and 3' untranslated region and the coding region (primers are listed in Table 1 according to sequence published by Chernoff et al. [18] [GenBank accession no. M31724]). Secondary PCRs were performed with incorporation of [α -³²P]dCTP on 2 μ l of the primary PCR in a total reaction volume of 25 μ l. In this way, the primary PCR was reamplified in seven overlapping secondary PCR segments ranging in size from 267 to 313 bp (primers listed in Table 1). All PCRs were performed in 25- μ l reaction volumes containing 1 \times PCR buffer, 0.2 μ mol/l of each primer, 0.2 mmol/l dNTP, 10 mCi/ml [α -³²P]dCTP, 0.3 units AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA), and 1.5 mmol/l MgCl₂. The cycling program was a denaturation step at 95°C for 2 min followed by 95°C for 30 s, annealing for 30 s (annealing temperature for each

primer set is given in Table 1), 72°C elongation for 30 s in 35 cycles, and finally an extension at 72°C for 9 min (except for the primary PCR, which was elongated for 2 min using a GeneAmp 9600 thermal cycler [Perkin Elmer]). The secondary PCR samples were analyzed by single-strand conformational polymorphism (SSCP) and heteroduplex analysis, applying two different experimental conditions as previously reported (19). The variants identified were sequenced on both strands using an ABI377 automated sequencer (Perkin Elmer) or by dideoxysequencing using the Thermo Sequenase cycle sequencing kit (USB, Cleveland, OH). All variants identified were confirmed on genomic DNA.

Genotyping. Genotyping of the P387L variant was carried out by PCR amplification of a 327-bp segment covering exon 9 on genomic DNA using the primers (5'-3' sense primer) CATCTCTGCCCTCTGATTC and (5'-3' antisense primer) TGAGACTGGCTCAGATGCAC (T_{anneal} 60°C and 2.0 mmol/l MgCl₂). The mutation removes a *Bst* I restriction enzyme site at position 101 in the segment. An additional control site is present at position 290 in the segment. Thus, enzyme digestion of PCR segments from wild-type carriers will produce three fragments: 37, 101, and 189 bp, whereas homozygous mutant carriers will produce only the 37-bp fragment and a 290-bp fragment. Heterozygous carriers will contain all fragments. Screening is performed by adding 5 units of *Bst* I enzyme (New England Biolabs, Beverly, MA) into 20 μ l of amplified PCR product, and after 4 h incubation at 55°C, products are loaded onto 3% agarose gels and visualized by staining with ethidium bromide.

Genotyping of the G381S variant was carried out by PCR amplification of a segment covering exon 9 on genomic DNA using the primers (5'-3' sense primer) TACCATCTCTGCCCTCT and (5'-3' antisense primer) GGTAGGATTCAGTTCTGTG (T_{anneal} 56°C and MgCl₂ 1.5 mmol/l) and PCR-SSCP and heteroduplex analysis. Genotyping of the 3'UTR+104insG variant was carried out by PCR amplification of a segment covering exon 10 and part of the 3'UTR region using the primers (5'-3' sense primer) GTCTGGGCTCATCTGAACTGT and (5'-3' antisense primer) GGACGGACGTTGGTTCTG (T_{anneal} 58°C and MgCl₂ 1.5 mmol/l) and by PCR-SSCP and heteroduplex analysis. SSCP genotyping revealed two intronic variants as described in RESULTS.

Statistical analysis. Fisher's exact test was applied to test for differences in allele and genotype frequencies. $P < 0.05$ was considered significant. SPSS software for windows (version 10.0) was used to carry out descriptive analysis and analysis using a generalized linear model. The analysis included age and BMI as covariate and sex and genotype as a fixed factor. The normal distribution of the residuals was visually verified. The odds ratios from the case-control study were calculated using logistic regression while controlling for age. For comparison of the quantification of the radioactive incorporation into peptides, a two-tailed distribution paired Student's *t* test was used on the log¹⁰-transformed data set from each of four independent assays. The values are expressed as means \pm SD.

In vitro peptide phosphorylation assay. Incorporation of [γ -³²P]ATP into wild-type peptide (RRRGAQAASPAKGE: 387P) and mutant peptide (RRRG AQAASLAKGE: 387L) by the p34 cell division cycle (p34^{cdc2}) kinase was performed in a final reaction mixture containing 50 μ l of 1 mmol/l of wild-type (387P) or mutant peptide (387L), 100 μ mol/l ATP at a final specific activity of

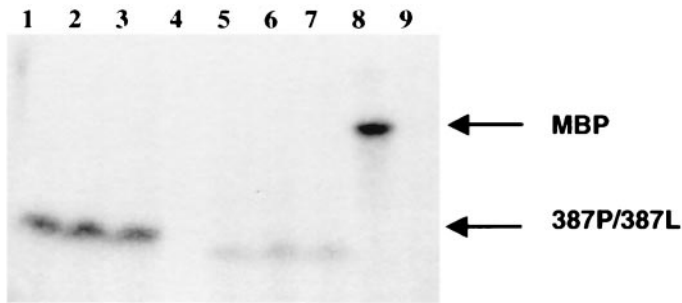


FIG. 1. In vitro peptide serine phosphorylation by p34^{cdc2} protein kinase. Equal amounts of wild-type and mutant peptide were loaded. Visualized are radioactive incorporation into wild-type (387P) and mutant (387L) peptide after gel exposure to a phosphoimager screen. Twenty-five µl 387P reactions (lanes 1-3); 15 µl BenchMark protein marker (lane 4); 25 µl 387L reactions (lanes 5-7); 25 µl MBP (lane 8; positive control); and 25 µl reaction mixture without p34^{cdc2} kinase added and with MBP as substrate (lane 9; negative control).

100 µCi/µmol [γ -³²P]ATP (Amersham Pharmacia Biotech), and reaction buffer containing 50 mmol/l Tris-HCl, 10 mmol/l MgCl₂, 2 mmol/l dithiothreitol, 1 mmol/l EGTA, 0.01% Brij 35, pH 7.5, and 0.272 units recombinant p34^{cdc2} protein kinase (New England Biolabs). The peptides were synthesized using Fmoc chemistry, and the resin-cleaved peptides were analyzed by mass spectral analysis (MALDI/TOF; Research Genetics, Invitrogen, Huntsville, AL). For negative and positive controls, a 0.33-µg/µl concentration of myelin basic protein (MBP; Sigma) was used in the final 50-µl reaction. The substrate and reaction mixtures were heated separately at 30°C for 1 min before mixing and then incubated at 30°C for 30 min. The reaction was stopped by the addition of 50 µl of 2 × Tricine SDS sample buffer (cat. no. LC1676; Novex, San Diego, CA) and 2.5% of 2-mercaptoethanol. The samples were subsequently heated for 2 min at 85°C, and then 25 µl of each sample was applied on a 16% tricine gel (Novex) and electrophoresed for 2 h at 110 V. The tricine gels were silver-stained to ensure peptide location using the protein silver stain kit PlusOne (Amersham Pharmacia Biotech). After gel exposure to phosphoimager screens, the radioactivity of the peptides was quantified by scanning in a Typhoon 8600 (Molecular Dynamics, Amersham Pharmacia Biotech). Quantification of the incorporation of [γ -³²P]ATP into wild-type and mutant peptide was examined in four independent assays (Fig. 1) using Image Quant 5.1 software (Molecular Dynamics, Amersham Pharmacia Biotech). The wild type from each assay was standardized to 100%, and the mutant was counted as a percentage of the wild type. SD is calculated from the log¹⁰-transformed data set (Fig. 2).

RESULTS

Mutation analysis of the PTP-1B gene. Primary analysis of the coding region of the PTP-1B gene, including the 5'-UTR from position -42 before translation start site and the 3'UTR to position +138 after the stop codon, revealed five variants. There were four silent variants, (NT CGA→CGG) R199R, (NT CCC→CCT) P303P, 3'UTR+104insG (insertion variant), and 3'UTR+86T→G (transversion variant), and one missense variant (NT CCA→CTA) P387L. Furthermore, during SSCP genotyping on genomic DNA using intronic primers, two missense variants, (NT GGT→AGT) G381S and (NT ACG→ATG) T420M, and two single-intron variants, IVS9+57C→T and IVS9+58G→A, were found. The T420M, 3'UTR+86T→G, IVS9+57C→T, and IVS9+58G→A variants were only found in one case each.

Association studies of the missense variants G381S and P387L and the insertion variant 3'UTR+104insG in type 2 diabetes. The prevalence of the G381S, P387L, and 3'UTR+104insG variants were further evaluated in a case-control study of type 2 diabetic patients and matched glucose-tolerant control subjects. As presented in Table 2, the allelic frequency of the G381S variant was 0.4% in type

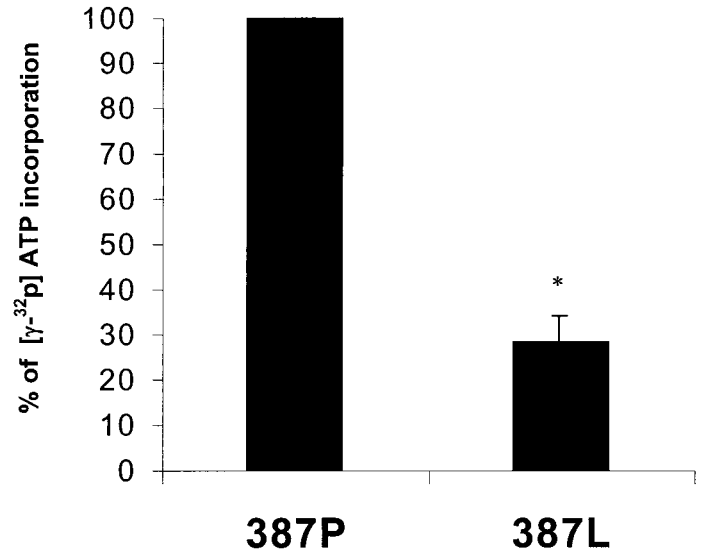


FIG. 2. Percentage incorporation of [γ -³²P]ATP by the p32^{cdc2} kinase into wild-type peptides (RRRGAQAASPAKGE: 387P) and mutant peptides (RRRGAQAASLAKGE: 387L). The figure represents a mean of four independent in vitro peptide phosphorylation assays quantified and corrected for background using ImageQuant 5.1 software. The wild type from each assay is 100%, and the mutant is counted as a percentage of the wild type, 387P = 100% and 387L = 28.4% ± 5.8. SD is calculated from the log¹⁰-transformed data set. **P* = 0.0012.

2 diabetic patients and 1.1% in control subjects (*P* = 0.143). The allelic frequency of the 3'UTR+104insG insertion variant was 6.9% among type 2 diabetic patients and 10.2% among control subjects (*P* = 0.20) (Table 2). The observed genotype frequencies of these variants were in Hardy-Weinberg equilibrium. Furthermore, the 3'UTR+104insG variant was not found to be associated with BMI, fasting serum insulin, or fasting plasma glucose among control subjects (data not shown).

The allelic frequency of the P387L variant was 1.4% (95% CI 0.4-2.4) among 527 type 2 diabetic patients and 0.5 (-0.1 to 1.1) among 542 glucose-tolerant control subjects. Thus, the P387L variant was significantly associated with type 2 diabetes (*P* = 0.037) (Table 2), with carriers having an age-corrected relative risk of type 2 diabetes of 3.7 (1.26-10.93, *P* = 0.02). When comparing heterozygous carriers of the P387L variant with the remaining group of wild-type carriers, the phenotypical and biochemical characteristics were not significantly different between the two groups (Table 3).

In vitro peptide phosphorylation assay. The P387L variant is situated next to a serine residue that is a target of the proline-directed p34^{cdc2} protein kinase. We investigated the incorporation of [γ -³²P]-labeled radioactivity into wild-type peptide (387P) versus mutant peptide (387L) in an in vitro kinase assay (Fig. 1). The assay results showed significantly lower p34^{cdc2} kinase proline-directed phosphorylation of the mutant compared with the wild-type peptides (387P: 100% vs. 387L: 28.4 ± 5.8%; *P* = 0.0012) (Fig. 2).

DISCUSSION

Considering the crucial role of PTP-1B in regulating insulin signaling and the clear physiological effect on insulin-stimulated glucose uptake associated with the lack of

TABLE 2

Genotype and allele frequencies of the G381S, P387L, and 3'UTR+104insG variants in PTP-1B and allelic frequencies in type 2 diabetic patients and glucose-tolerant control subjects

	G381S		P387L		3' UTR + 104insG	
	Diabetic patients	Control subjects	Diabetic patients	Control subjects	Diabetic patients	Control subjects
<i>n</i>	527	530	527	542	490	499
Gly/Gly	523	522	—	—	—	—
Gly/Ser	4	8	—	—	—	—
Pro/Pro	—	—	513	537	—	—
Pro/Leu	—	—	14	5	—	—
Wild type	—	—	—	—	426	416
Heterozygous	—	—	—	—	60	81
Homozygous	—	—	—	—	4	2
Allelic frequency	0.4	1.1	1.4	0.5	6.9	10.2
95% CI	-1 to 0.9	-0.2 to 2.0	0.4-2.4	-0.1 to 1.1	5.0-9.0	7.3-13.1
<i>P</i>	0.143		0.037		0.20	

P values are based on Fisher's exact test of genotypic distribution.

PTP-1B in mice, PTP-1B is a credible candidate to be involved in the assumed polygenic basis of type 2 diabetes in humans. In addition, PTP-1B is located on the chromosomal region 20q13.1-13.2 on the long arm of chromosome 20, which has been linked to quantitative trait loci for obesity and fasting serum insulin levels (20) as well as type 2 diabetes (21). In the present study, we have identified a rare P387L variant of the PTP-1B gene that is associated with type 2 diabetes in the Danish population and with impaired in vitro serine phosphorylation of a PTP-1B peptide. We found that the variant has an almost three times higher prevalence among type 2 diabetic patients, conferring an effective relative risk of 3.7 for carriers of this variant.

The proline at position 387 in the COOH-terminal regulatory region of the protein is conserved between mouse, rat, and humans and, in the human sequence, is located next to a serine residue (position 386). It is part of a

consensus sequence known to be phosphorylated in vitro by the proline-directed kinase p34^{cdc2} (22,23), in vivo in states of mitosis (24,25) and, perhaps more importantly, in response to various stress stimuli (26). Replacing the proline by a leucine reduced the phosphorylation of the serine by 70% in our in vitro studies of the relevant peptide. Testing the replacement on the NetPhos 2.0 Phosphorylation Prediction database further confirmed that the predicted likelihood of serine 386 being a phosphorylation target was reduced from 0.884 to 0.135 (range from 0 to 1.0) when replacing proline 387 with leucine (27).

Because a lack of PTP-1B is associated with increased insulin sensitivity, a PTP-1B variant associated with type 2 diabetes would be hypothesized to cause increased PTP-1B levels and phosphatase activity. However, the direct consequences on enzyme function of the P387L variant is still unclear, and Ser386 phosphorylation can be associated with both increased (24) as well as decreased

TABLE 3

Clinical and biochemical characteristics of type 2 diabetic patients and age-matched glucose-tolerant control subjects classified according to genotype of the P387L variant of the PTP-1B gene

	Control subjects			Diabetic patients		
	P387P	P387L	<i>P</i>	P387P	P387L	<i>P</i>
<i>n</i> (M/F)	254/283	4/1		296/218	11/3	
Age (year)	56 (9)	60 (0)	0.4	60 (11)	64 (10)	0.2
Age at diagnosis (year)	—	—	—	50 (11)	60 (10)	0.5
BMI (kg/m ²)	25.8 (3.8)	27.9 (3.2)	0.2	29.1 (5.2)	27.7 (4.5)	0.5
Waist/hip ratio	0.88 (0.10)	0.85 (0.07)	0.5	0.94 (0.009)	0.96 (0.005)	0.8
HbA _{1c} (%)	—	—	—	8.0 (1.6)	8.0 (1.4)	1.0
Fasting plasma glucose (mmol/l)	5.1 (0.5)	5.1 (0.3)	0.8	9.7 (3.3)	10.3 (3.5)	0.5
Fasting serum C-peptide (pmol/l)	562 (220)	521 (152)	0.7	683 (297)	564 (297)	0.3
Fasting serum insulin (pmol/l)	40 (21)	33 (15)	0.5	77 (57)	57 (37)	0.4
Blood pressure						
Systolic (mmHg)	—	—	—	147 (24)	146 (23)	0.5
Diastolic (mmHg)	—	—	—	84 (11)	85 (8)	0.6
Fasting serum triglycerides (mmol/l)	1.2 (0.6)	1.1 (0.3)	0.5	2.1 (1.6)	1.9 (0.9)	0.7
Fasting serum cholesterol (mmol/l)	5.9 (1.0)	5.7 (0.6)	0.7	5.8 (1.2)	5.8 (0.8)	0.7
Fasting serum HDL (mmol/l)	1.5 (0.4)	1.5 (0.2)	0.7	1.2 (0.3)	1.1 (0.2)	0.3
Treatment (%)						
Diet	—	—	—	28	27	
OHA	—	—	—	57	64	
Insulin	—	—	—	15	9	

Data are means ± SD. *P* values are adjusted for BMI, sex, and age and are given for differences between wild-type and heterozygous diabetic patients. OHA, oral hyperglycemic agent.

(25) PTP-1B enzymatic activity. Moreover, human studies have also shown conflicting results in relating PTP-1B mRNA levels and phosphatase activity to insulin sensitivity and obesity (10,28–31), and in vitro studies suggest that the expression levels of PTPases may not be the primary determinant of their insulin receptor phosphorylation activity (32), indicating that other factors may regulate PTPase activity. This is also suggested by the surprising localization of the PTP-1B to the endoplasmic reticulum by a COOH-terminal motif (7). Therefore, it is likely that the in vivo action of the PTP-1B protein is regulated by both the phosphorylation status as well as by restricted accessibility to intracellular substrates, and it might be hypothesized that the P387L variant and potentially the phosphorylation status at position Ser386 may affect the activity or localization of the protein. Thus, a recent study has shown that increased phosphoserine content of PTP-1B in vivo was followed by increased PTP-1B activity and indicated that insulin and protein kinase A have a critical role in the regulation of PTP-1B (33). However, the mechanisms of COOH-terminal PTP-1B regulation have not been fully elucidated, and further studies are needed to evaluate the functional consequences of the P387L variant.

The search for variability in the human PTP-1B gene also identified several additional variants, of which only the silent variant P303P has been previously identified and reported to have no association with type 2 diabetes (34). The association study of the missense variant G381S and the insertion variant 3'UTR+104insG showed no significant association with type 2 diabetes, although the 3'UTR+104insG variant shows a trend toward an association. These findings need to be tested in larger association studies.

In conclusion, we identified a rare P387L variant in the PTP-1B gene that was associated with type 2 diabetes in the examined Danish population. The variant peptide exhibited reduced in vitro serine phosphorylation. Testing for association of the variant to diabetes in other populations as well as further studies of the effect of the variant on PTP-1B function are needed.

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