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Association of Protein Tyrosine Phosphatase 1B (PTPN1) Gene Polymorphisms (1023C>A and 467T>C) With Type 2 Diabetes: A Case-Control Study

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Abstract

Background: Type 2 diabetes (T2D) is a heterogeneous disorder that results from a combination of environmental and genetic factors. Insulin resistance (IR) is the core defect in T2D. The molecular mechanisms underlying IR are poorly understood. Protein tyrosine kinases and Protein tyrosine phosphatase 1B (*PTPN1*) are important regulators of insulin signal transduction. The association of *PTPN1*single-nucleotide polymorphisms (SNPs) with traits related to T2D has been investigated. The aim of this study was to determine the association of 1023C>A and 467T>C gene polymorphisms with Type 2 diabetes and its related metabolic traits.

M**ethod:** Polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) analyses were carried out to detect the 1023C>A and 467T>C variants of *PTPN1* gene in 100 Egyptian patients with T2D as compared to controls (n=80).

Results: The 1023C>A *PTPN1*genotype was significantly associated with T2DM (X^2=7.816, P=0.02). The C allele was more frequent in the T2DM as compared to controls (p=0.001), odds ratio (OR) and 95% CI= 0.282 (0.131-0.606). We did not observe any significant difference in 467T>C *PTPN1* genotypes between patients and control groups (X^2=2.205, P=0.332). 1023C>A and 467T>C *PTPN1* variants showed non-significant association with diabetic metabolic traits in both groups; plasma insulin levels, fasting blood glucose levels (FBG), HOMA-IR, the lipid profile parameters , diastolic blood pressure (DBP), systolic blood pressure (SBP), Waist circumference (WC) and body mass index (BMI).

Conclusion: The *PTPN1* promoter variant 1023C>A was associated with presence of T2D, but it had no correlation with any of neither metabolic traits nor obesity in this study but we could not detect any association between 467T>C variants of *PTPN1* gene with T2D Egyptian patients nor related traits in this study. Further studies must be done on a larger population to detect any potential metabolic association.

Keywords: Diabetes mellitus; Insulin resistance; Protein; Tyrosine phosphatase; Gene; Polymorphisms

Introduction

Diabetes mellitus (DM) is the eighth most frequent disease leading cause of death throughout the world and now ranks the fifth, following communicable diseases, cardiovascular disease, cancer, and injuries [1]. Prevalence of diabetes mellitus is increasing worldwide [2]. More than 300 million people are suffering from DM all over the world and studies show that population aging, changes in lifestyle and improvement in detection techniques are most important factors in increasing the numbers of cases [3]. The link between IR and type-2 diabetes has been recognized for over half a century. IR is not important only as the most powerful predictor of future development of type 2 diabetes; it is also a therapeutic target of hyperglycemia [4].

The molecular mechanisms of IR are till now not completely explained but defects in the signal transduction pathway downstream of the insulin receptor sounds to be included [5]. Protein tyrosine kinases and protein tyrosine phosphatases are important regulators of insulin signal transduction [5]. Protein tyrosine kinases and protein tyrosine phosphatases play role in regulation of insulin signal transduction [6]. Nowadays, more attention has been focused on protein tyrosine phosphatase, non-receptor type 1 (**PTPN1**) [7]. The **PTPN1** gene encodes protein tyrosine phosphatase enzyme (PTB)-1B (EC 3.3.3.48), which down regulates the insulin signaling cascade binds the insulin receptor via dephosphorylation of phosphotyrosine residues of the activated insulin receptor [8,9]. Mice deficient for **PTPN1** display enhanced insulin sensitivity and resistance to diet-induced obesity [10], as well as general leanness due to an increased basal metabolic rate [11].

In vitro inhibition of PTP-1B improves insulin sensitivity [12,13]. These functional features, together with its genomic location under the chromosome 20q13 type 2 diabetes linkage, support **PTPN1** as a candidate gene influencing susceptibility to IR and type 2 diabetes [14]. Multiple studies have examined the association of **PTPN1** single-nucleotide polymorphisms (SNPs) with traits related to T2D and they have demonstrated that *PTPN1* expression increases in obese individuals and those with T2D [15,16]. The effect of genetic variations on gene expression is one of the most likely mechanisms by which such

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variations can contribute to complex diseases such as T2D [6].

To our Knowledge till now no reported studies have been made to identify variants within the *PTPN1* promoter region and investigate their relationships with T2D in Egypt. In the present study, we aimed to examine the effect of **PTPN1** genetic variations (1023C>A and 467T>C) on susceptibility to T2D by comparing the **PTPN1** gene (1023C>A and 467T>C) alleles and genotypes between T2D and healthy Egyptian subjects. Moreover , to investigate the effects of these polymorphisms on insulin sensitivity (FPI, FPG , HbA1c , HOMA) , obesity markers (BMI,WC) , hypertension (SBP, DBP) and also on different quantitative metabolic parameters as total lipid profile parameters (TC, TG, HDL-C and LDL-C) .

Subjects and Method

Subjects, blood pressure and anthropometric measurements

This case–control study was started in February 2014 to December 2015. It included 180 subjects. They were recruited from Endocrinology outpatient clinics of the Internal Medicine Department, Zagazig University hospitals. All subjects were Egyptians from Sharkia–Egypt and they belonged to the same ethnic group. A written informed consent was obtained from all patients before enrollment in the study. The study was approved by Zagazig University ′s ethics committee. Type 2 diabetes was defined by the 1999 criteria of the World Health Organization (fasting blood glucose level >126 mg/dl and/or 2-h postprandial blood glucose level >200 mg/dl) (World Health Organization, 2006). Patients who did not meet these criteria as under treatment but who gave a history of T2DM were also included in the study.

Subjects were classified into two main groups:

- **Group I "control group":** 80 normal volunteers (38 females and 42 males). Their mean ages were ranged from 37–65 years with a mean value ± S.D of 47.16 ± S.D of 6.72 years .Who had been matched for BMI, sex, age and socioeconomic background, they had no evidence of DM, hypertension, obesity, hypercholesterolemia, family history or previous history of stroke or transient ischemic attacks and smoking on the basis of their clinical history and physical examination.

- **Group II "Type 2 diabetic patients":** included 100 patients (53 males and 47 females), aged from 34-68 with a mean value ± S.D of 49.23 ± S.D of 7.58 years. Body mass index [BMI = weight (kg)/height $(m)^2$] was calculated. Waist circumference (WC) was measured while the subjects were standing up, with a tape placed at the midpoint level between the lower intercostal border and the anterior superior iliac supine while the subject was gently exhaling.

-There was no statistical difference (P>0.05) regarding age and sex among the groups.

Biochemical analyses of blood samples

Sample collection: Overnight fasting venous blood samples were collected from the subjects in EDTA containing tubes using standardized protocol and equipment., separated into two samples one whole blood for DNA extraction and *PTPN1* gene SNPs detection and the measurement of glycated hemoglobin (HbA1c) [17] .The other plasma specimen was used for measuring total lipid profile parameters, plasma insulin level and FBG. Other basic biochemical blood tests were measured by standard chemical and enzymatic commercial methods in the Medical Biochemistry department and hospital laboratories.

Laboratory investigations, including:

Determination of HbA1c in blood [17].

Fasting plasma glucose levels (FPG) according to Trinder [18] using glucose enzymatic (GODPAP)- liquizyme Kits (Biotechnology, Egypt).

Lipid profile: plasma levels of total cholesterol (TC), Triglyceride (TG), and HDL-C [19]. LDL cholesterol (LDL-C) was measured According to Friedewald et al. LDL was calculated as follows: LDL=TC-HDL-TG\5 [20].

Fasting plasma insulin (FPI) By enzyme amplified sensitivity immunoassay according to Starr et al**.** [21] using KAP1251-INSEASIA (Enzyme Amplified Sensitivity Immunoassay) Kits (BioSource Europe S.A., Belgium).

HOMA-IR: homeostasis model assessment (where HOMA= (fasting insulin (μU/ml) × fasting plasma glucose (mg/dl) /405 [22].

Genotyping

Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit purchased from Promega. After extraction, the quality of the extracted material was visualized in 1% agarose gel and the concentration was obtained by a spectrophotometer. -1023C>A SNP -467T>C variants were genotyped using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). Primer sequences (Iontec, Bioron). Amplification reactions of 1023C>A and 467T>C variants of *PTPN1* gene were set up separately for and polymorphic sites of *promotor* gene [6] (Table 1).

The PCR was done using Taq PCR Master Mix kit (Qiagen, GmbH) as following: 25 ml of Taq PCR master mix was dispensed into each PCR tube, and then the following materials were added to each tube containing 100 ng of extracted DNA, 25 mM forward primer, and 25 mM reverse primer (Operon Biotechnologies, Inc.) and then 19 ml dd H_2O was added giving a final volume of 50 ml. Following initial denaturation at 94ºC for 5 min, amplification was performed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58 °C for 30 sec -1023C>A SNP and annealing of -467T>C SNP at 57°C for 30 sec extension at 72°C for 30 sec. Final extension was allowed to proceed at 72°C for 5 min; 8 ml of the PCR products were digested overnight at 65°C with 10 U *Bcl*I at 37°C and for 3 h with 10 U *Ava*I enzyme for -1023C>A and -467T>C SNPs , respectively .The digested PCR products were resolved on 3% agarose gels stained with ethidium bromide.

Polymorphisms	Primers	Annealing Temp (°C)	PCR-RFLP products
-1023C>A SNP	Forward: 5'TAGCAG AAA CCG AGT TTC ACC -3' Reverse: 5'-CCTGGG TAA CAG AAT CAG ACC -3'	58°C	CC- 312-bp CA -312-249- 63-bp AA-249- 63-bp
-467T>C SNP	Forward 5'-TTC ATT CCTGCA GCA CCC AAG-3' Reverse: 5'-GTT GAG TCACAG AGT GAG TGG-3'	57°C	CC-269-bp segment. CT-269, 163, and 106 bp. TT-163 and 106 bp

Table 1: PCR-RFLP pattern of (1023C>A and 467T>C) of PTPN1 gene polymorphisms.

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS 16.0, SPSS Inc., Chicago, Illinois, USA). Descriptive data were expressed as mean and standard deviation (SD). The differences between mean value for each parameter between controls and diabetic patients were tested by student's "*t*" test. One-way analysis of variance (ANOVA, F test) was used to examine the variation in different metabolic and anthropometric variables with the genotypes .To determine whether any significant differences in polymorphisms frequencies occurred between the case and the control populations the allele and genotype frequencies were compared, using the Chi square (X^2) method. Where significant *P*-values were generated, the odds ratio (OR) was calculated. Associations between the disease and genotypes were assessed by calculating odds ratios and 95 confidence intervals (CI). Statistical significance was assumed when p values were < 0.05.

Results

Demographic, clinical, and laboratory characteristics of the all studied groups are summarized in Table 2

All studied biomarkers were significantly higher in diabetic patients when compared to non-diabetic controls. Except for HDL-C which showed significant decreased levels (P<0.05) (Table 2).

Distribution of 1023C>A and 467T>C of *PTPN1* Gene polymorphisms

The allele and genotype frequency distribution and carriage rate of 1023C>A and 467T>C *PTPN1* Gene among patients and controls were shown in Table 3 and Figure 1. The results showed significant differences between diabetic patients regarding the genotype and allele distributions of the 1023C>A of *PTPN1* Gene polymorphisms. The CC genotype for the 1023C>A was significantly more frequent in diabetics than controls (X^2=7.816, P=0.02). The C allele was more frequent in the diabetic group as compared to controls (X^2=11.545, P=0.001), odds ratio (OR) and 95% CI for the C allele of 1023C>A=0.282 (0.131-0.606). Otherwise we could not find a significant association between diabetic patients and controls regarding the 467T>C *PTPN1* genotype and allele distributions (X^2=2.205, P=0.332 and X^2=0.015, P=0.528) odds ratio (OR) and 95% CI=0.953 (0.442-2.054).

In addition, we also compared the 1023C>A and 467T>C *PTPN1* Gene genotypes with different biochemical and clinical phenotypes in the diabetic and control groups separately. No significant differences (*P*>0.05) in anthropometric or biochemical features were observed between the wild-type and heterozygous individuals at1023C>A and

Parameters	Controls (n=80)	Diabetic group (n=100)	t-test)	P value
Age (years)	47.16 ± 6.72	49.23 ± 7.58	-1.909	P=0.058
Sex	38 F (47.5 %) 42M (52.5 %)	47 F (47%) 53 M (53%)	X^2=0.004	P=0.533
FPI (µU/ml)	16.91 ± 2.75	29.34 ± 6.42	-16.17	P=0.000
FPG (mg/dl)	100.27 ± 9.62	139.16 ± 8.18	-29.273	P=0.000
HbA1c %	5.72 ± 1.34	11.93 ± 2.28	-21.472	P=0.000
HOMA-IR	4.24 ± 0.94	10.12 ± 2.5	-19.89	P=0.000
TC (mg/dl)	200.86 ± 19.46	312.52 ± 70.11	-13.81	P=0.000
TG (mg/dl)	141.58 ± 15.46	194.94 ± 36.18	-12.31	P=0.000
LDL-C (mg/dl)	105.51 ± 10.76	234.97 ± 25.56	-42.37	P=0.000
HDL-C (mg/dl)	53.66 ± 9.41	41.68 ± 5.19	10.84	P=0.000
SBP (mmHg)	110.4 ± 12.4	125.45 ± 13.78	-7.61	P=0.000
DBP (mmHg)	76.68 ± 8.11	85.7 ± 10.94	-6.14	P=0.000
WC (cm)	90.7 ± 9.07	103.19 ± 8.37	-9.57	P=0.000
BMI (kg/m^2)	21.37 ± 2.55	30.79 ± 3.18	-21.45	P=0.000

FPI: Fasting Plasma Insulin; FPG: Fasting Plasma Glucose; Hba1c: Glycated Hemoglobin; TC: Total Cholesterol; TG: Triglyceride; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High Density Lipoprotein-Cholesterol; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WC: Waist Circumference; BMI: Body Mass Index; X^2= Chi–Square For Non-Parametric Values.

Table 2: Basic characteristics of all participant groups.

	1023 C>A			467 T>C		
Genotype	Groups		Genotype	Groups	
	Group I (N=80) N (%)	Group II (N=100) N (%)		Group I (N=80) N (%)	Group II (N=100) N (%)
CC	58 (72.5 %)	88 (88.0 %)	TT	68 (85.0%)	92 (92.0%)
CA	15 (18.8 %)	10 (10.0%)	TC	9 (11.2 %)	6 (6.0 %)
AA	7 (8.8 %)	2 (2.0%)	CC	3 (3.8 %)	2 (2.0 %)
X^2 P - value	7.816 0.02		X^2 P-value	2.205 0.332 (NS)	
	N (%)	N (%)		N (%)	N (%)
C allele	131 (81.88 %)	186 (93.0 %)	T allele	145 (90.63%)	190 (95.0 %)
A allele	29 (18.12%)	14 (7.0 %)	C allele	15 (9.37 %)	10 (5.0 %)
X^2 P OR (95%CI)	11.575 0.001 0.282 (0.136-0.606)		X^2 P OR (95%CI)	0.015 0.528 0.953 (0.442-2.054)	

Table 3: Genotype and Allele frequency for PTPN1gene polymorphisms at 1023C>A and 467T>C in all studied groups.

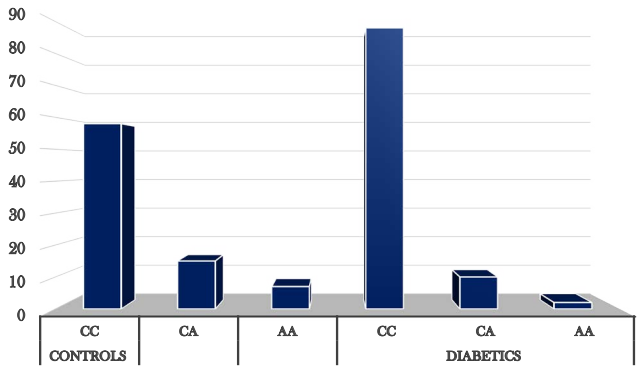

Figure 1: Genotype and allele frequency for PTPN1gene polymorphisms at 1023C>A and 467T>C in all studied groups.

467T>C *PTPN1* variants in the T2D group or controls, data is shown in Tables 4 and 5.

Discussion

T2D is a heterogeneous disorder which can be affected by a combination of environmental and genetic factors [23] that contribute to its pathogenesis by influencing B-cell function and tissue insulin sensitivity [6]. In 2013 it was reported that in Middle East region about 35 million people suffered from diabetes [24]. The prevalence of diabetes has been estimated as 382 million people throughout the world while nearly 176 million of them seem to be still undiagnosed [24]. It is predicted that this prevalence reaches to 592 million by 2035 [24]. Insulin resistance (IR) is the core defect in T2D; it plays an important role in the development of such abnormalities as impaired glucose tolerance, type 2 diabetes, obesity, and hyperlipidemia [25].

PTPN1 is a negative regulator of the insulin signaling pathway [26]. The role of PTPN1 in multiple crucial metabolic pathways has been illustrated in mice deficient for PTPN1 [27]. These investigations suggest that PTPN1 plays a role in attenuating insulin signal transduction [28]. Variations in the PTPN1 promoter might affect gene regulation and could therefore be associated with T2D [28]. Much attention has been directed toward PTPN1, which regulate the phosphorylation of insulin receptor negatively [6]. The effect of genetic polymorphisms on gene expression is considered to contribute to metabolic diseases such as T2D, and PTPN1gene SNPs can affect gene expression and contribute to traits in health and disease. To our knowledge until now no researches have been made to identify variants within the *PTPN1* promoter region and examine their relationships with T2D and its metabolic related traits on Egyptian population.

In this study, we examined the effect of **PTPN1** genetic variations (1023C>A and 467T>C) on susceptibility to T2D by comparing the **PTPN1** gene (1023C>A and 467T>C) alleles and genotypes between T2D patients and healthy Egyptian subjects. Moreover , we examined the effects of those polymorphisms on insulin sensitivity (FPI, FPG , HbA1c, HOMA), obesity markers (BMI,WC), hypertension (SBP, DBP) and also on different quantitative metabolic parameters as total lipid profile parameters (TC, TG, HDL-C and LDL-C) .

Our results showed significant differences between diabetic patients regarding the genotype and allele distributions of the 1023C>A of PTPN1 Gene polymorphism. The CC genotype and The C allele for the 1023C>A were significantly more frequent in diabetic Egyptian patients when compared to controls. Otherwise we could not find a significant association between diabetic patients and controls regarding the 467T>C PTPN1 genotype and allele distributions. These results are in agreement with the results of Meshkani et al. [6] on Iranian subjects who reported that 1023C>A SNP significantly differed between the T2D and non-diabetic individuals (P<0.020), whereas the none of the

Parameters	1023C>A Genotypes				467T>C Genotypes			
	CC (n=58)	CA (n=15)	AA (n=7)	Anova-F value P-value	TT (n=68)	TC (n=9)	CC (n=3)	Anova -F value P-value
FPI (µU/ml)	16.76 ± 2.52	17.0 ± 2.11	18.24 ± 3.5	1.07 0.348	16.84 ± 2.62	18.36 ± 3.38	14.16 ± 0.84	2.92 0.06
FPG (mg/dl)	100.97 ± 9.5	96.33 ± 11.44	103.0 ± 11.03	1.57 0.215	100.35 ± 9.9	101.11 ± 8.31	96.0 ± 8.18	0.326 0.723
HbA1c %	5.6 ± 1.32	5.33 ± 1.1	5.17 ± 0.677	0.632 0.534	5.76 ± 1.35	5.37 ± 1.38	5.96 ± 1.45	0.366 0.695
HOMA-IR	4.22 ± 0.87	4.07 ± 1.02	4.34 ± 0.89	0.249 0.780	4.22 ± 0.89	4.31 ± 1.27	4.5 ± 1.11	0.151 0.860
TC (mg/dl)	199.71 ± 2.71	205.94 ± 3.7	199.1 ± 6.5	0.65 0.527	200.4 ± 20.24	200.6 ± 14.7	211.6 ± 13.9	0.468 0.628
TG(mg/dl)	142.6 ± 16.55	148.8 ± 23.8	136.2 ± 11.34	1.333 0.270	142.1 ± 16.3	139.8 ± 9.99	135.5 ± 8.54	0.325 0.724
LDL-C(mg/dl)	107.4 ± 1.37	101.73 ± 3.0	109.3 ± 2.95	1.977 0.145	105.77 ± 1.34	102.82 ± 3.2	107.67 ± 3.2	0.355 0.703
HDL-C(mg/dl)	54.61 ± 9.95	55.3 ± 13.99	63.92 ± 16.2	2.1 0.13	54.3 ± 9.5	51.84 ± 9.1	45.0 ± 2.35	1.616 0.205
SBP (mmHg)	111.2 ± 12.32	115.73 ± 10.8	117.3 ± 10.9	1.472 0.236	111.35 ± 12.1	106.1 ± 14.1	101.6 ± 10.4	1.51 0.228
DBP (mmHg)	77.58 ± 7.8	76.33 ± 6.93	77.85 ± 6.98	0.176 0.84	76.98 ± 8.11	76.66 ± 8.66	70.0 ± 5.0	1.06 0.349
WC(cm)	91.37 ± 9.2	91.9 ± 6.81	88.85 ± 9.14	0.306 0.737	91.11 ± 8.85	89.97 ± 11.23	83.66 ± 6.35	1.00 0.373
BMI (Kg /m^2)	21.42 ± 2.61	19.97 ± 1.94	20.42 ± 1.96	2.34 0.103	21.43 ± 2.57	21.26 ± 2.9	20.36 ± 0.68	0.256 0.775

FPI: Fasting Plasma Insulin; FPG: Fasting Plasma Glucose; Hba1c: Glycated Hemoglobin; TC: Total Cholesterol; TG: Triglyceride; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High Density Lipoprotein-Cholesterol; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WC: Waist Circumference; BMI: Body Mass Index

Table 4: Different clinical and anthropometric parameters with different PTPN1gene polymorphisms at 1023C>A and 467T>C in control group.

Parameters	1023C>A Genotypes				467T>C Genotypes			
	CC (n=88)	CA (n=10)	AA (n=2)	Anova (F-value) P- value	AA (n=68)	AG (N=9)	GG (n=3)	Anova (F-value) P -value
FPI (µU/ml)	27.34 ± 5.77	31.04 ± 3.85	26.25 ± 3.74	2.017 0.139	28.91 ± 6.4	34.62 ± 4.44	33.6 ± 7.21	2.767 0.068
FPG (mg/dl)	138.71 ± 7.9	138.29 ± 9.53	140.55 ± 4.03	0.065 0.937	139.11 ± 8.3	139.3 ± 7.65	141.05 ± 7.9	0.055 0.947
HbA1c %	11.8 ± 2.37	12.41 ± 1.68	14.1 ± 0.71	1.211 0.302	11.86 ± 2.32	12.16 ± 1.3	14.6 ± 1.97	1.451 0.239
HOMA	9.31 ± 2.28	11.1 ± 1.99	10.7 ± 0.98	3.01 0.054	9.96 ± 0.265	11.99 ± 0.304	11.65 ± 0.21	0.108 2.278
TC (mg/dl)	349.56 ± 6.3	342.6 ± 16.55	434.6 ± 29.0	0.125 2.12	310.1 ± 70.4	348.43 ± 51.84	317.85 ± 117.9	0.847 0.432
TG (mg/dl)	211.11 ± 3.2	191.36 ± 15.3	230.5 ± 16.2	2.113 0.126	192.73 ± 36.41	226.73 ± 16.55	201.15 ± 31.9	2.598 0.08
LDL-C (mg/dl)	243.66 ± 36.88	249.9 ± 56.03	275.65 ± 52.25	0.74 0.48	233.78 ± 2.7	249.3 ± 6.42	246.85 ± 17.15	1.26 0.288
HDL-C (mg/dl)	42.45 ± 0.62	39.26 ± 1.46	40.0 ± 1.5	1.558 0.216	41.77 ± 5.31	41.68 ± 3.42	37.5 ± 1.41	0.658 0.520
SBP (mmHg)	125.3 ± 15.05	133.5 ± 7.1	132.5 ± 10.6	1.640 0.199	124.62 ± 13.7	135.8 ± 10.68	132.5 ± 10.6	2.182 0.118
DBP (mmHg)	106.2 ± 0.82	114.4 ± 2.89	112.0 ± 2.0	2.422 0.094	85.1 ± 11.01	91.66 ± 6.05	95.0 ± 14.14	1.777 0.175
WC (cm)	105.6 ± 0.89	109.9 ± 2.26	107.6 ± 8.8	1.276 0.284	102.74 ± 8.3	109.9 ± 8.89	103.6 ± 4.31	2.115 0.126
BMI (Kg/m^2)	31.64 ± 4.01	33.75 ± 3.96	29.65 ± 0.77	1.556 0.216	30.8 ± 3.26	30.2 ± 2.53	30.9 ± 0.424	0.122 0.885

FPI: Fasting Plasma Insulin; FPG: Fasting Plasma Glucose; Hba1c: Glycated Hemoglobin; TC: Total Cholesterol; TG: Triglyceride; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High Density Lipoprotein-Cholesterol; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WC: Waist Circumference; BMI: Body Mass Index

Table 5: Different clinical and anthropometric parameters with different PTPN1gene polymorphisms at 1023C>A and 467T>C in diabetic group.

other PTPN1 gene SNPs were significantly associated with T2D.

Meta-analysis study including 7883 individuals from three European case-control samples (from US, Poland and Scandinavia) did not detect the association for any single PTPN1 SNP or haplotype with T2D [29]. Echwald et al. [30] investigated a rare Pro→Leu change at position 387 of PNTP1 and reported the association with T2D in a Danish population. Otherwise Weng et al [31], Santaniemi et al [32] and Wanic et al [33] could not find any association in a Chinese or a Finnish population and Polish, respectively. On the other hand a more extensive evaluation of noncoding genetic variants at this locus has been reported in two non-overlapping case/control samples; Bento et al. [34] found that several SNPs were associated with T2D. In addition, we also compared the 1023C>A and 467T>C PTPN1 Gene genotypes with different biochemical and clinical phenotypes in the diabetic and control groups separately. No significant differences (P>0.05) regarding anthropometric or biochemical features were observed between the wild-type and heterozygous individuals at1023C>A and 467T>C PTPN1 variants in the T2D group or healthy subjects, that was in good accordance with Meshkani et al. [6].

However, several other studies which examined the association of PTPN1 SNPs with T2D metabolic traits have reported a significant association as in Mok et al. [35] study who reported an association between an SNP in exon 8 and impaired glucose tolerance and T2D in Canadian aboriginal individuals. Echwald et al. [30] noticed an association of a coding SNP (P387L) with T2D in Danes. Paola et al. [36] recognized a nucleotide insertion in the 3′ untranslated region (UTR) that was associated with IR in obese individuals [34,37] investigated common variants in PTPN1 and found association with T2D and IR.

Moreover, our T2D association results strongly differ from those obtained in Caucasian American populations, the Bento et al. [34] and Palmer et al. [37] in Hispanic Americans from the Insulin Resistance Atherosclerosis Study Family Study (IRASFS) IRASFS, who revealed significant association of PTP- 1B gene polymorphisms with metabolic traits of T2D. The Bento et al. [34] and Palmer et al. [37] investigations displaying associations to T2D were performed in American subjects, whereas the Florez et al. [29] study and the study of Cheyssac et al. [38] were focused on European populations.

However, our association analysis of metabolic syndrome quantitative traits does not support the hypothesis of a possible impact of **PTPN1** genetic variations (1023C>A and 467T>C) on insulin sensitivity, HOMA index and hypertension which are characteristics of the metabolic syndrome. We could not find significant association between PTPN1 gene two SNPs (1023C>A and 467 T>C) and obesity markers, neither BMI nor WC. In accordance with our results, Echwald et al. [30] could not find association between **PTPN1** genetic variations with type 2 diabetes, BMI, fasting glucose, or fasting insulin in the Danish sample or a large Swedish sample of 2,309 non-diabetic subjects [39]. In contrary, Cheyssac et al [38] showed a weak association between SNP rs914458 and moderate obesity (p=0.04) and a trend towards association with severe obesity for SNP rs6126033 located in the first intron (p=0.05), his findings supported the association of the **PTPN1** genetic SNPs with insulin sensitivity and characteristics of the metabolic syndrome. Paola et al [36] reported an association of a common insertion of a guanosine at position 1484 of the 3′ untranslated region (UTR) (1484insG) with features of the metabolic syndrome in two Italian samples comprising 812 normoglycemic individuals [36]. Spencer-Jones et al [40] reported several associations between **PTPN1**gene variants and insulin sensitivity quantitative traits.

In the present study diabetic patients showed significant increase in TC , TG , LDL-C plasma levels and significant decrease in HDL-C levels when compared to controls but without significant association with **PTPN1** genetic variations 1023C>A or 467T>C .This disagreed

with Kipfer-Coudreau et al [41] who detected an association between **PTPN1** genetic variation and dyslipidemia in the French population. An association was also being reported between the Pro387Leu variant and hypertriglyceridemia in a German population [42]. Moreover, Olivier et al. recorded associations of **PTPN1** gene variants with BMI and TC level in an Asian population [43]. These results can be referred to the known role of **PTPN1** in the dephosphorylation of the JAK2 kinase, which is vital step in the leptin signalling pathway, and in the regulation of the expression of the lipogenic genes [44]. These different results question the impact of the **PTPN1** gene variations on the risk of T2D in populations of different ethnic origin. These divergent findings could be due to heterogeneity of T2D etiology among the different populations, perhaps driven by differences in genetic or environmental modifiers. Further adjustment of patient phenotypes, matching for all possible factors on increased sample size, will be needed to examine the possible effect of **PTPN1** gene SNPs on type 2 diabetes and related metabolic traits.

Conclusion

Our present observations permit us to suggest that the *PTPN1* promoter variant 1023C>A was associated with presence of T2D and could be one of the genetic factors predisposing to development type 2 diabetic in Egyptian population. But it had no correlation with any of neither metabolic traits nor obesity in this study. However, we could not detect any association between 467T>C variants of *PTPN1* gene with T2D Egyptian patients nor metabolic related traits. Further studies must be done on a larger population size to detect any potential metabolic association between different *PTPN1* gene variants and T2D with its metabolic traits.

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References

1. Chu H ,Wang M, Zhong D, shi D, Ma L, et al (2013) Adipo Q polymorphisms are associated with type 2 diabetes mellitus: a meta-analysis study. Diabetes/ Metabolism Research and Reviews 29: 532–545.
2. IDF Diabetes Atlas, New Estimates for 2012 of Diabetes Prevalence, Mortality, and Healthcare Expenditures, 5th edition (2012).
3. International Diabetes Federation, IDF Diabetes Atlas, International Diabetes Federation, 6th edition (2013) http://www.idf.org/diabetesatlas.
4. Taylor R (2012) Insulin resistance and type 2 diabetes. Diabetes 61: 778-779.
5. Goldstein BJ, Bittner-Kowalczyk A, White MF, Harbeck M (2000) Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B: possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. J Biol Chem 275: 4283-4289.
6. Meshkani R, Taghikhani M, Al-Kateb H, Larijani B, Khatami S, et al (2007) Polymorphisms within the Protein Tyrosine Phosphatase 1B (PTPN1) Gene Promoter: Functional Characterization and Association with Type 2 Diabetes and Related Metabolic Traits. Clinical Chemistry September 53: 1585-1592.
7. Iselius L, Lindsten J, Morton NE, EfendiÄ‡ S, Cerasi E, et al. (1985) Genetic regulation of the kinetics of glucose-induced insulin release in man. Studies in families with diabetic and non-diabetic probands. Clin Genet 28: 8-15.
8. Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, et al. (1996) Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. Diabetes 45: 1379-1385.
9. Goldstein BJ (2002) Protein-tyrosine phosphatases: emerging targets for therapeutic intervention in type 2 diabetes and related states of insulin resistance. J Clin Endocrinol Metab 87: 2474–2480.
10. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, et al. (1999) Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 283: 1544-1548.
11. Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, et al. (2000) Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 20: 5479–5489.
12. Xie L, Lee SY, Andersen JN, Waters S, Shen K, et al. (2003) Cellular effects of small molecule PTP1B inhibitors on insulin signaling. Biochemistry 42: 12792-12804.
13. Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, et al. (2004) Allosteric inhibition of protein tyrosine phosphatase 1B. Nat Struct Mol Biol 11: 730-737.
14. Florez JC, Hirschhorn J, Altshuler D (2003) The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. Annu Rev Genomics Hum Genet 4: 257-291.
15. McGuire MC, Fields RM, Nyomba BL, Raz I, Bogardus C, et al. (1991) Abnormal regulation of protein tyrosine phosphatase activities in skeletal muscle of insulin-resistant humans. Diabetes 40: 939–942.
16. Ahmad F, Azevedo JL, Cortright R, Dohm GL, Goldstein BJ (1998) Alterations in skeletal muscle protein-tyrosine phosphatase activity and expression in insulin-resistance human obesity and diabetes. J Clin Invest 100: 449–458.
17. Abraham EC, Huff TA, Cope ND, Wilson JB Jr, Bransome ED Jr, et al. (1978) Determination of the glycosylated hemoglobin (HB AI) with a new micro column procedure. Suitability of the technique for assessing the clinical management of diabetes mellitus. Diabetes 27: 931-937.
18. Trinder P (1969) Enzymatic determination of glucose. An Clin Bioch 6: 24-27.
19. Assmann G, Schriewer H, Schmitz G, Hägele EO (1983) Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/ $MgCl_2$. Clin Chem 29: 2026-2030.
20. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499-502.
21. Starr JI, Mako ME, Juhn D, Rubenstein AH (1978) Measurement of serum proinsulin-like material: cross-reactivity of porcine and human proinsulin in the insulin radioimmunoassay. J Lab Clin Med 91: 683-692.
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419.
23. Hamman RF (1992) Genetic and environmental determinants of non-insulin-dependent diabetes mellitus (NIDDM). Diabetes Metab Rev 8: 287-338.
24. IDF Diabetes Atlas, New Estimates for 2012 of Diabetes Prevalence, Mortality, and Healthcare Expenditures, 5th edition, 2012, http://www.idf.org/.)
25. BoduÅ‚a A, Wdowczyk M, Adamiec R (2005) [The role of protein tyrosine phosphatase (PTP-1B) in insulin resistance]. Postepy Hig Med Dosw (Online) 59: 203-207.
26. McGuire MC, Fields RM, Nyomba BL, Raz I, Bogardus C, et al. (1991) Abnormal regulation of protein tyrosine phosphatase activities in skeletal muscle of insulin-resistant humans. Diabetes 40: 939-942.
27. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, et al. (1999) Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 283: 1544-1548.
28. Kenner KA, Anyanwu E, Olefsky JM, Kusari J (1996) Protein-tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signaling. J Biol Chem 271: 19810-19816.
29. Florez JC, Agapakis CM, Burtt NP, Sun M, Almgren P, et al. (2005) Association testing of the protein tyrosine phosphatase 1B gene (PTPN1) with type 2 diabetes in 7,883 people. Diabetes 54: 1884-1891.
30. Echwald SM, Bach H, Vestergaard H, Richelsen B, Kristensen K, et al. (2002) 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*. Diabetes 51: 1-6.
31. Weng J, Yan J, Huang Z, Sui Y, Xiu L (2003) Missense mutation of Pro387Leu in protein tyrosine phosphatase-1B (PTP-1B) is not associated with type 2 diabetes in a Chinese Han population. Diabetes Care 26: 2957.

32. Santaniemi M, Ukkola O, Kesäniemi YA (2004) Tyrosine phosphatase 1B and leptin receptor genes and their interaction in type 2 diabetes. J Intern Med 256: 48-55.

33. Wanic K, Malecki MT, Klupa T, Warram JH, Sieradzki J, et al. (2007) Lack of association between polymorphisms in the gene encoding protein tyrosine phosphatase 1B (PTPN1) and risk of Type 2 diabetes. Diabet Med 24: 650-655.

34. Bento JL, Palmer ND, Mychaleckyj JC, Lange LA, Langefeld CD, et al. (2004) Association of protein tyrosine phosphatase 1B gene polymorphisms with type 2 diabetes. Diabetes 53: 3007-3012.

35. Mok A, Cao H, Zinman B, Hanley AJ, Harris SB, et al. (2002) A single nucleotide polymorphism in protein tyrosine phosphatase PTP-1B is associated with protection from diabetes or impaired glucose tolerance in Oji-Cree. J Clin Endocrinol Metab 87: 724-727.

36. Di Paola R, Frittitta L, Miscio G, Bozzali M, Baratta R, et al. (2002) A variation in 3' UTR of hPTP1B increases specific gene expression and associates with insulin resistance. Am J Hum Genet 70: 806-812.

37. Palmer ND, Bento JL, Mychaleckyj JC, Langefeld CD, Campbell JK, et al. (2004) Association of protein tyrosine phosphatase 1B gene polymorphisms with measures of glucose homeostasis in Hispanic Americans: the insulin resistance atherosclerosis study (IRAS) family study. Diabetes 53: 3013–3019.

38. Cheyssac C, Lecoeur C, Dechaume A, Bibi A, Charpentier G, et al. (2006) Analysis of common PTPN1 gene variants in type 2 diabetes, obesity and associated phenotypes in the French population. BMC Med Genet 7: 44.

39. Dahlman I, Wahrenberg H, Persson L, Arner P (2004) No association of reported functional protein tyrosine phosphatase 1B 3' UTR gene polymorphism with features of the metabolic syndrome in a Swedish population. J Intern Med 255: 694–695.

40. Spencer-Jones NJ, Wang X, Snieder H, Spector TD, Carter ND, et al. (2005) 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. Diabetes 54: 3296-304.

41. Kipfer-Coudreau S, Eberlé D, Sahbatou M, Bonhomme A, Guy-Grand B, et al. (2004) Single nucleotide polymorphisms of protein tyrosine phosphatase 1B gene are associated with obesity in morbidly obese French subjects. Diabetologia 47: 1278-1284.

42. Gouni-Berthold I, Giannakidou E, Müller-Wieland D, Faust M, Kotzka J, et al. (2005) The Pro387Leu variant of protein tyrosine phosphatase-1B is not associated with diabetes mellitus type 2 in a German population. J Intern Med 257: 272-280.

43. Olivier M, Hsiung CA, Chuang LM, Ho LT, Ting CT, Bustos VI, et al. (2004) Single nucleotide polymorphisms in protein tyrosine phosphatase 1beta (PTPN1) are associated with essential hypertension and obesity. Hum Mol Genet 13: 1885-1892.

44. Rondinone CM, Trevillyan JM, Clampit J, Gum RJ, Berg C, et al. (2002) Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. Diabetes 51: 2405-2411.