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Protein Tyrosine Phosphatase 1B (*PTPN1*) Gene polymorphism (467T>C) and Metabolic Syndrome: A Pilot Study

Amal MH Mackawy*

Assistant Professor, Medical Biochemistry and Molecular biology, Medical Laboratory Department, Applied Medical Science College, Qassim University, Saudi Arabia
Assistant Professor, Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Zagazig University, Egypt

**Corresponding e-mail: amalmackawy@hotmail.com*

ABSTRACT

Background: *The metabolic syndrome (Met S) is composed of heart attack predisposing factors as diabetes, abdominal obesity, high cholesterol, and high blood pressure. Protein tyrosine phosphatase 1B (PTP1B), is negatively regulating the leptin and insulin signalling, with positive correlation with adiposity and contributes to insulin resistance. The effect of PTP1B on the obesity is still vague. This study aimed to study the association of PTPN1 polymorphism (467T>C) with Met S components and its metabolic compartments via examining the PTPN1 (467T>C) alleles and genotypes between Met S Egyptian patients and controls.* ***Methods:*** *Polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) analyses were applied to investigate the 467T>C variants in 100 Egyptian Obese patients comparing to 80 controls.* ***Results:*** *No significant association was observed of 467T>C PTPN1 genotypes between patients and healthy Egyptians (X^2=0.674, P=0.714). 467T> C PTPN1 variants displayed a non-significant correlation with Met S components.* ***Conclusion:*** *The PTPN1 promoter variant of 467T C was not associated with Met S components and T2DM metabolic related traits in type 2 diabetic Egyptian patients. More studies are required on a larger scale to examine any potential metabolic association.*

Keywords: Metabolic syndrome, Obesity, Diabetes mellitus, Insulin resistance, Protein tyrosine phosphatase, Gene polymorphisms

INTRODUCTION

The metabolic syndrome (Met S) constitutes group of clinical phenotypes, including central obesity, hypertension, hyperglycaemia, and dyslipidaemia. The metabolic syndrome (Met S) is a commonly encountered cluster of clinical phenotypes, including central obesity, hypertension, hyperglycaemia, and dyslipidaemia. Identifying genetic determinants of Met S will lead to better understanding of its progression and pathogenesis [1]. The prevalence of obesity in Egypt and the Middle East countries have been increased [2]. According to the World Health Organization, obesity in the Eastern Mediterranean countries became noticeable, most of young age Egyptian females are considered to be obese [2]. Lifestyle and dietary habits among Egyptians have been recently changed to increase the prevalence of overweight and obesity in a very short time. However, many researches are highlighting on the phenomenon of body weight gain and obesity in the well-developed societies, with the appearance of obesity in developing countries that is little unexplained and understood [3]. White adipose tissue (WAT) is the main storage site of unneeded dietary energy, and has a crucial effect on the body glucose homeostasis [4]. Diabetes mellitus (DM) is the 8th disease causing high morbidity throughout the world with increasing to be the fifth recently, coming after contagious diseases, cardiovascular morbidities, malignancies, and traumas [5]. The prevalence of DM is mounting all over the world [6]. The correlation between the insulin resistance (IR) and type 2 diabetes has been widely identified. IR is not substantial only in predicting the occurrence of T2DM, it is also considered to be a target of hyperglycaemia therapy [7]. The detailed intracellular pathology of IR is not fully explained; however, the defect of the insulin receptor signal transduction pathway may be accused [5]. Protein tyrosine kinases and protein tyrosine phosphatases are vital modifiers of insulin transcellular signalling cascade [8,9]. Recently, more studies have been acting on clarifying the

role of protein tyrosine phosphatase, non-receptor type 1 (*PTPN1*) on glucose homeostasis through IR and insulin sensitivity [10]. Protein tyrosine phosphatase enzyme (*PTB*)-*1B* (EC3.3.3.48), which downregulates the insulin signalling cascade via de-phosphorylation of phosphotyrosine residues of the activated insulin receptor [11,12]. Mice deficient for *PTPN1* exhibited augmented insulin sensitivity and decreased diet-induced obesity [13], as well as general slimness with enhanced basal metabolic rate [14]. Experimental laboratory tests on suppression of PTP-1B augments insulin sensitivity [15,16]. Combined these functional characters and its genomic locus under chromosome 20q13 T2DM linkage, shore the *PTPN1* as a possible responsible gene affecting the predisposition to IR and type 2 DM [17].

Several researches have investigated the correlation of *PTPN1* single-nucleotide polymorphisms (SNPs) with T2DM and its metabolic elements and they have elucidated that *PTPN1* expression enhances in obese people and in T2DM [18,19].

The impact of genetic diversity on *PTPN1* gene expression is one of the most likely possibility to implicate on complex diseases like T2DM [9]. The present study aimed to investigate the impact of *PTPN1* genetic SNP (467T> C) on susceptibility to Met S compartments in T2DM by comparing the *PTPN1* gene (467T>C) alleles and genotypes between diabetic patients with met S and Egyptian healthy controls. In addition, to test the influence of this polymorphism on obesity indicators (Waist circumference (WC), body mass index (BMI)), insulin sensitivity (fasting blood glucose levels (FBG), HOMA-IR, fasting plasma insulin (FPI), glycated haemoglobin A1c (HbA1c)), hypertension (diastolic blood pressure (DBP) and systolic blood pressure (SBP) and also on different quantitative metabolic parameters as total lipid profile parameters (TC, TG, H[2D] L-C, and LDL-C).

SUBJECTS AND METHODS

This case control study was conducted from June 2014 to December 2015. It is comprised of 180 subjects. They were recruited from Endocrinology outpatient clinics of the Internal Medicine Department, Zagazig University hospitals. All subjects were Egyptians from El-Sharkia province Egypt. Informed oral and written consent was obtained before participating in the study. The study was approved by Zagazig University's ethics committee.

Met S was diagnosed as per NCEP-ATP III criteria: WC ≥102 cm for men, ≥ 88 cm for women; FBG ≥110 mg/dl; triglycerides (TG) ≥150 mg/dl; HDL-cholesterol 40 mg/dl for males, 50 mg/dl for females; SBP ≥130 mmHg or DBP≥ 85 mmHg [20].

Type 2 diabetes was identified by the World Health Organization (WHO) criteria (fasting blood glucose level 126 mg/dl and/or 2-h postprandial blood glucose level 200 mg/dl) (World Health Organization, 2006). Obesity was determined on the basis of the BMI more than 26 kg/m^2. BMI was computed as weight (kg) divided by squared height (m^2).

Subjects were classified into two main groups: Group I "control group ": composed of 80 healthy controls (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. They were not complaining from T2DM, hypertension, obesity, or dyslipidaemia.

They were non-smokers, moreover had no family history or past history of stroke or transient ischemic attacks.

Group II " diabetic patients with Met S: 51 males and 49 females were included, aged from 38-65 with a mean value ± S.D of 51.45 ± 9.37 years. Body mass index was calculated. WC was measured on by letting the subject standing up, then placing the tape at the midpoint level between the lower intercostal border and the anterior superior iliac supine and asked the subject to exhale gently.

Age and sex variations among the groups revealed a non-statistical significance (t= -2.909, P=0.064) (X^2= 0.040, P=0.480), respectively (Table 2).

Collection of blood sample

EDTA- containing tubes were used for collecting fasting venous blood samples from the subjects, then the blood samples were divided into two samples one whole blood for DNA extraction, and *PTPN1* gene SNP examination and for glycated haemoglobin (HbA1c) estimation [21]. The other plasma sample was used for estimation the total lipid profile parameters, plasma insulin level, and FBG. Standard chemical and enzymatic commercial methods were applied for measuring other basic biochemical blood tests in the Medical Biochemistry department and hospital laboratories.

Laboratory investigations

1) FPG according to Trinder [22] using glucose enzymatic (GODPAP) - liquizyme Kits (Biotechnology, Egypt).

2) Measurement of HbA1c in blood [21].

3) Lipid profile: total cholesterol (TC), Triglyceride (TG), and HDL-C [23]. LDL -cholesterol (LDL-C) was calculated as follows: LDL=TC-HDL-TG\5 [24].

4) Estimation of fasting plasma insulin (FPI) using KAP1251-INSEASIA (Enzyme Amplified Sensitivity Immunoassay) [0K] its (BioSource Europe S.A., Belgium) [25].

5) HOMA-IR: homeostasis model assessment (where HOMA= (fasting insulin (μU/ml) x.

6) Fasting plasma glucose (mg/dl) /405 [26].

Genotyping

Wizard Genomic DNA Purification Kit (Promega) was applied for DNA was isolation.

Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was applied for genotyping the 467T> C alleles and variants. All the PCR conditions with PCR-RFLP pattern of 467T> C of *PTPN1* SNP are listed in the Table 1 [10]. About 25 ml of Taq PCR master mix (Qiagen, GmbH) in each PCR tube containing 100 ng of extracted DNA, followed by 25 mM forward primer, and 25 mM reverse primer (Operon Biotechnologies, Inc.) and then ddH_2O was added giving a final volume of 50 ml. Initial denaturation step was at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec extension at 72°C for 30 sec. Final extension step was adjusted at 72°C for 5 min; about 8 ml to 10 ml of the PCR products were digested at 37°C for 24 h with 10 U Ava I restriction enzyme -467T C SNPs. About 3% agarose gels stained with ethidium bromide was used for detection the digested PCR products.

Table 1 PCR-RFLP pattern of (467T>C) of *PTPN1* gene polymorphisms

	Primer sequences	Annealing Temperature (°C)	PCR-RFLP products
-467T>C SNP	Forward 5'-TTC ATT CCTGCA GCA CCC AAG-3'	57°C	CC-269-bp segment
	Reverse: 5'-GTT GAG TCACAG AGT GAG TGG-3'		CT -269, 163, and 106 bp
			TT-163 and 106 bp

The statistical analysis was conducted by applying the software program (SPSS 16.0, SPSS Inc., Chicago, Illinois, USA). Descriptive data were expressed as the mean ± standard deviation (SD). Student's t-test was used to test the differences of the mean values between controls and metabolic syndrome affected diabetic patients. One-way analysis of variance (ANOVA, F-test) was used to examine the statistical differences of the metabolic and anthropometric variables with the variant genotypes. Chi- square (X^2) test was applied to examine the variations of the gene alleles frequencies between the diseased and controls. Where significant p-values were generated, the odds ratio (OR) was calculated. Odds ratios and 95 confidence intervals (CI) were calculated to examine the association between the disease and genotypes. P-values less than 0.05 are considered to be significant.

RESULTS

Basic characteristics of the cases and controls are summarized in Table 2.

All studied biomarkers were significantly increased in T2DM patients with metabolic syndrome when compared

to non-diabetic controls. HDL-C levels were significantly decreased in patients than controls (P<0.05) (Table 2).

Distribution of 467T> C of *PTPN1* gene polymorphism:

The allele and genotype frequency of 467T C *PTPN1* gene in patients and controls were illustrated in Table 3 and Figure 1.

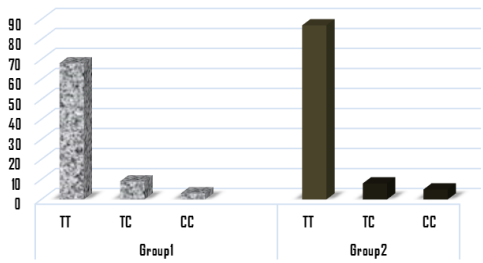

Figure 1 Genotype and Allele frequency for *PTPN1* gene polymorphisms at 467T>C in all studied groups

Table 2 Basic characteristics of all participant groups

Parameters	Control (n=80)	Diabetic with Met S group (n=100)	t-test	P value
Age (years)	47.16 ± 6.72	51.45 ± 9.34	-2.909	P=0.064
Sex	38 F (47.5%)	49 F (49%)	X^2= 0.040	P=0.480
	42 M (52.5%)	51 M (51%)		
FPI (µU/ml)	16.91 ± 2.75	28.24 ± 6.40	-14.78	P=0.000
FPG (mg/dl)	100.27 ± 9.62	140.69 ± 8.46	-26.643	P=0.000
HbA1c%	5.72 ± 1.34	12.08 ± 2.3	-24.995	P=0.000
HOMA-IR	4.24 ± 0.94	10.65 ± 2.38	-20.95	P=0.000
TC (mg/dl)	200.86 ± 19.46	321.38 ± 68.25	-15.013	P=0.000
TG (mg/dl)	141.58 ± 15.46	230.3 ± 53.49	-13.84	P=0.000
LDL-C (mg/dl)	105.51 ± 10 .76	244.37 ± 31.14	-39.87	P=0.000
HDL-C (mg/dl)	53.66 ± 9.41	39.27 ± 5.1	12.113	P=0.000
SBP (mmHg)	110.4 ± 12.4	132.24 ± 10.28	-11.756	P=0.000
DBP (mmHg)	76.68 ± 8.11	94.47 ± 18.51	-9.6	P=0.000
WC (cm)	90.7 ± 9.07	108.47 ± 10.3	-11.1	P=0.000
BMI (kg/m^2)	21.37 ± 2.55	32.15 ± 3.16	-23.134	P=0.000

FPI: Fasting plasma insulin; FPG: Fasting plasma glucose; HbA1c: Glycated haemoglobin; 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 3 Genotype and Allele frequency for *PTPN1* gene polymorphisms at 467T>C in all studied groups

Genotype	Groups	
	Group I (N=80) N (%)	Group II (N=100) N (%)
TT	68 (85.0%)	87 (87.0%)
TC	9 (11.2%)	8 (8.0%)
CC	3 (3.8%)	5 (5.0%)
X^2	0.674	
P-value	0.714 (NS)	
Genotype	N (%)	N (%)
T allele	145 (90.63%)	182 (91.0%)
C allele	15 (9.37%)	18 (9.0%)
X^2	0.015	
P	0.522	
OR (95%CI)	0.96 (0.466-1.962)	

The present results could not detect any significant correlation between patients with Met S and controls regarding the 467T C *PTPN1* genotype and allele distributions.

Table 4 Different clinical and anthropometric parameters with different *PTPN1* gene polymorphisms at 467T>C in control group

Parameters	467T>C Genotypes			ANOVA F-value P-value
	TT (n=68)	TC (n=9)	CC (n=3)	
FPI (µU/ml)	16.84 ± 2.62	18.36 ± 3.38	14.16 ± 0.84	F=2.92
				P=0.06
FPG (mg/dl)	100.35 ± 9.9	101.11 ± 8.31	96.0 ± 8.18	F=0.326
				P=0.723
HbA1c%	5.76 ± 1.35	5.37 ± 1.38	5.96 ± 1.45	F=0.366
				P=0.695
HOMA-IR	4.22 ± 0.89	4.31 ± 1.27	4.5 ± 1.11	F=0.151
				P=0.86
TC (mg/dl)	200.4 ± 20.24	200.6 ± 14.7	211.6 ± 13.9	F=0.468
				P=0.628
TG(mg/dl)	142.1 ± 16.3	139.8 ± 9.99	135.5 ± 8.54	F=0.325
				P=0.724
LDL-C(mg/dl)	105.77 ± 1.34	102.82 ± 3.2	107.67 ± 3.2	F=0.355
				P=0.703

HDL-C(mg/dl)	54.3 ± 9.5	51.84 ± 9.1	45.0 ± 2.35	F=1.616
				P=0.205
SBP (mmHg)	111.35 ± 12.1	106.1 ± 14.1	101.6 ± 10.4	F=1.51
				P=0.228
DBP (mmHg)	76.98 ± 8.11	76.66 ± 8.66	70.0 ± 5.0	F=1.06
				P=0.349
WC (cm)	91.11 ± 8.85	89.97 ± 11.23	83.66 ± 6.35	F=1
				P=0.373
BMI (Kg/m^2)	21.43 ± 2.57	21.26 ± 2.9	20.36 ± 0.68	F=0.256
				P=0.775

(X^2 =0.674, P=0.74 and X^2 =0.015, P=0.522) odds ratio (OR) and 95% CI = 0.96 (0.466-1.962). In addition, on comparing the 467T>C *PTPN1* genotypes with biochemical and clinical parameters in the Met S patients and controls, non-significant differences (P>0.05) were detected. Data is shown in Tables 4 and 5.

Table 5 Different clinical and anthropometric parameters with different *PTPN1* gene polymorphisms at 467T>C in diabetic patients with Met S

Parameters	467T>C Genotypes			Anova (F-value) P -value
	TT (n= 87)	TC (n=8)	CC (n=5)	
FPI (µU/ml)	28.76 ± 6.41	29.93 ± 3.95	35.64 ± 4.6	F=2.957
				P=0.057
FPG (mg/dl)	140.34 ± 8.44	137.89 ± 8.52	138.7 ± 5.0	F=0.386
				P=0.681
HbA1c%	12.17 ± 2.24	12.83 ± 1.43	13.38 ± 2.23	F=0.978
				P=0.38
HOMA	10.78 ± 2.37	11.9 ± 1.14	12.83 ± 1.05	F=2.642
				P=0.076
TC (mg/dl)	326.1 ± 69.2	341. 94 ± 54.52	351.58 ± 77.04	F=2.578
				P=0.232
TG (mg/dl)	230.25 ± 51.41	229. 22 ± 12.9	242.1 ± 43.54	F=0.14
				P=0.87
LDL-C (mg/dl)	258.47 ± 35.53	264.0 ± 35.76	270.7 ± 18.54	F=1.63
				P=0.167
HDL-C (mg/dl)	39.5 ± 5.28	40.05 ± 4.61	37.42 ± 4.07	F=0.442
				P=0. 644
SBP (mmHg)	132.4 ± 10.03	136.88 ± 9.61	132.0 ± 7.58	F=0.76
				P=0.47
DBP (mmHg)	95.2 ± 16.16	101.16 ± 16.89	109.6 ± 14.5	F=2.325
				P=0.103
WC (cm)	107.87 ± 10.13	113.1 ± 7.17	107.16 ± 9.23	F=1.071
				P=0.347
BMI (Kg/m^2)	32.3 ± 3.26	33.18 ± 2.95	31.92 ± 3.78	F=0.32
				P=0.727

DISCUSSION

Several definitions of the Met S exist, and it is confused if the risk factors having a higher cardiovascular risk [27]. Met S has confirmed to link the obesity, IR, and related traits in relation to cardiovascular disease risk. Furthermore, the Met S as defined by the National Cholesterol Education Program sounds to be heritable, that may point to a genetic basis [27]. The prevalence of diabetes has been increased to reach near half a million all over the world, about 35 million people in Middle East region suffered from diabetes in the year of 2013 and expected to increase to 592 million by the year of 2035 [28]. IR plays a vital role in the generation of Met S components such as glucose intolerance, type 2 diabetes, central obesity, and dyslipidaemia [29].

PTPN1 regulates negatively the insulin signalling pathway in skeletal muscles [30]. *PTPN1* effects on several metabolic pathways have been discovered in *PTPN1* deficient mice [31]. These multiple research studies opened the door for the negative effect of the *PTPN1* on the insulin signal transduction [32].

The effects of *PTPN1* promoter polymorphisms on the gene regulation may strengthen the link of that gene with

the T2DM disease, obesity, and Met S [32]. Interestingly many types of research have been directed toward *PTPN1* regulation on the insulin receptor phosphorylation, trying to clarify the effect of *PTPN1* on insulin sensitivity [9]. *PTPN1* gene SNPs may influence the gene expression that correlates the SNPs in that gene to be associated with some metabolic disorders as T2DM and metabolic syndrome [33].

The aim of the current research was to examine the relationship between the (467T C) *PTPN1SNP* and Met S components by analyzing the *PTPN1* gene (467T C) alleles and genotypes in T2D patients with Met S and in healthy Egyptians. The study examined also the effects of that SNP on the insulin sensitivity (FPI, FPG, HbA1c, and HOMA index), 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).

The current study could not reveal significant correlation among patients with Met S and the healthy volunteers for the 467T C *PTPN1* genotype and allele variants. Meshkani, et al. [10] results were in harmony with the present findings, who stated that none of the *PTPN1* gene SNPs was correlated with T2DM except that of 1023C A SNP.

In agreement with this study results, meta-analysis study included 7883 Europeans in three case-control studies, did not reveal any association for *PTPN1* SNPs or haplotype with T2DM [33].

In our previous study of Mackawy, et al. [34] we investigated the association of *PTPN1* gene polymorphisms (1023C >A and 467T >C) with Type 2 diabetic Egyptian patients and concluded that *PTPN1*1023C>A may have association with T2DM, but not correlated with any of metabolic traits and obesity. Moreover, in Mackawy, et al. study the 467T>C variants were not associated with T2DM.

The current results also are in line with those of Weng, et al. [35], Santaniemi, et al. [36] and Wanic, et al. [37] who did not determine any association in a Chinese or a Finnish and Polish population, respectively.

In contrary, Bento, et al. [38] had discovered associations between T2DM and several *PTPN1* SNPs at this locus. Furthermore, this study failed to find any significant association between anthropometric or biochemical parameters with the homozygous and heterozygous individuals at 467T C *PTPN1* variants in the T2DM with Met S patients or healthy controls. This finding was in harmony with Meshkani, et al. [10].

On contrary, other articles have detected a significant association of *PTPN1* SNPs with T2DM metabolic traits as in Mok, et al. [39] who discovered an association between an SNP in exon 8 with decreased glucose tolerance and T2DM in Canadian aboriginal individuals. Paola, et al. [40] reported significant association with IR in obese Italian individuals. Other studies of Bento, et al. [38] and Palmer, et al. [41] had found associations with T2DM and R SNPs.

Bento, et al. [38] and Palmer, et al. [41] reported different results among Hispanic Americans from the Insulin Resistance Atherosclerosis Study Family Study (IRASFS) IRASFS, they found a significant association of PTP-1B gene polymorphisms with metabolic traits of T2D. Moreover, Florez, et al. [33] and the Cheyssac, et al. [42] discovered that significant association but in European populations.

The present study results did not match with the hypothesis of the possible impact of 467T C *PTPN1* SNP on Met S traits such as insulin sensitivity, HOMA-I, and hypertension which are biomarkers of the metabolic syndrome.

No significant correlation between 467 T>C SNP and obesity was detected in the current study. In agreement with present study results was the study of Echwald, et al. [43] who recorded no association between *PTPN1* SNPs and metabolic syndrome traits in the Danish or Swedish diabetic subjects.

Oppositely, Cheyssac, et al. [42] displayed a correlation between *PTPN1* gene SNPs rs914458 and rs6126033 with both moderate and severe obesity, respectively. Those findings supported the corporation of the *PTPN1* genetic SNPs with insulin sensitivity and metabolic syndrome traits. In addition, multiple correlations between *PTPN1* gene variants and insulin sensitivity traits were observed by Spencer-Jones, et al. [44].

The 467T> C SNP in the current findings did not display any significant association with Met S parameters. This was in disagreement with Kipfer-Coudreau, et al. [45] who confirmed that association with dyslipidaemia in the French population. An association was also found between the Pro387Leu variant and increase in blood glucose levels in a German population [46]. Associations of *PTPN1* gene variants with BMI and TC level in an Asian population were observed by Olivier, et al. [47]. The findings of all those studies can be explained by the dephosphorylation effect of *PTPN1* on the JAK2 kinase that is a crucial step expression of the lipogenic genes [48].

Those variable results open the window for the effect of the *PTPN1* gene SNPs on the risk of Met S in T2DM in subjects of different ethnic origin. These conflict results could be owed to heterogeneity of Met S origin among the different populations, most probable caused by variations in genetic or environmental alternates. No single gene or cluster of genes has been responsible for Met S among different populations, likely due to the complex interaction between gene and environment necessary for expression of this phenotype. Further amendment of patient characterization on larger sample size, will be required to analyze the impact of *PTPN1* gene SNPs on Met S parameters in T2DM and related metabolic characters.

CONCLUSION

The present study could not detect any association between 467T C variants of *PTPN1* gene with T2DM Egyptian patients nor metabolic related traits. Ongoing future studies are required on larger sample size to discover any possible metabolic correlation between different *PTPN1* gene variants and metabolic syndrome.

DECLARATIONS

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Conflict of interest

There are no conflicts of Interest.

REFERENCES

[1] Lahiry, Piya, Rebecca L. Pollex, and Robert A. Hegele. "Uncloaking the genetic determinants of metabolic syndrome." *Journal of Nutrigenetics and Nutrigenomics* 1.3 (2008): 118-125.

[2] Musaiger, Abdulrahman O. "Overweight and obesity in the Eastern Mediterranean Region: can we control it?" (2004).

[3] Lfotouh, Abo, et al. "Central obesity among adults in Egypt: prevalence and associated morbidity." (2008).

[4] Herman, Mark A., and Barbara B. Kahn. "Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony." *Journal of Clinical Investigation* 116.7 (2006): 1767.

[5] Chu, Haiyan, et al. "ADIPOQ polymorphisms are associated with type 2 diabetes mellitus: A meta-analysis study." *Diabetes/Metabolism Research and Reviews* 29.7 (2013): 532-545.

[6] IDF Diabetes Atlas, New estimates for 2012 of diabetes prevalence, mortality, and healthcare expenditures, 5th edition (2012). International Diabetes Federation, IDF Diabetes Atlas, International Diabetes Federation, 6th edition (2013).

[7] Taylor, Roy. "Insulin resistance and type 2 diabetes." *Diabetes* 61.4 (2012): 778-779.

[8] Goldstein, Barry J., et al. "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." *Journal of Biological Chemistry* 275.6 (2000): 4283-4289.

[9] Meshkani, Reza, et al. "Polymorphisms within the protein tyrosine phosphatase 1B (*PTPN1*) gene promoter: functional characterization and association with type 2 diabetes and related metabolic traits." *Clinical Chemistry* 53.9 (2007): 1585-1592.

[10] Iselius, L., et al. "Genetic regulation of the kinetics of glucose-induced insulin release in man Studies in families with diabetic and non-diabetic probands." *Clinical Genetics* 28.1 (1985): 8-15.

[11] Seely, B. Lynn, et al. "Protein tyrosine phosphatase 1B interacts with the activated insulin receptor." *Diabetes* 45.10 (1996): 1379-1385.

[12] Goldstein, Barry J. "Protein-tyrosine phosphatases: Emerging targets for therapeutic intervention in type 2 diabetes and related states of insulin resistance." *The Journal of Clinical Endocrinology & Metabolism* 87.6 (2002): 2474-2480.

[13] Elchebly, Mounib, et al. "Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene." *Science* 283.5407 (1999): 1544-1548.

[14] Klaman, Lori D., et al. "Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice." *Molecular and Cellular Biology* 20.15 (2000): 5479-5489.

[15] Xie, Laiping, et al. "Cellular effects of small molecule PTP1B inhibitors on insulin signaling." *Biochemistry* 42.44 (2003): 12792-12804.

[16] Wiesmann, Christian, et al. "Allosteric inhibition of protein tyrosine phosphatase 1B." *Nature Structural & Molecular Biology* 11.8 (2004): 730-737.

[17] Florez, Jose C., Joel Hirschhorn, and David Altshuler. "The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits." *Annual Review of Genomics and Human Genetics* 4.1 (2003): 257-291.

[18] McGuire, Mary C., et al. "Abnormal regulation of protein tyrosine phosphatase activities in skeletal muscle of insulin-resistant humans." *Diabetes* 40.7 (1991): 939-942.

[19] Grundy, Scott M., et al. "Diagnosis and management of the metabolic syndrome." *Circulation* 112.17 (2005): 2735-2752.

[20] Abraham, E. C., et al. "Determination of the glycosylated hemoglobins (Hb AI) with a new microcolumn procedure: suitability of the technique for assessing the clinical management of diabetes mellitus." *Diabetes* 27.9 (1978): 931-937.

[21] Trinder, P. "Enzymatic colorimetric method for glucose determination." *Ann Clin Biochem* 6 (1969): 24-27.

[22] Assmann, Gerd, et al. "Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl2." *Clinical Chemistry* 29.12 (1983): 2026-2030.

[23] Friedewald, William T., Robert I. Levy, and Donald S. Fredrickson. "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge." *Clinical Chemistry* 18.6 (1972): 499-502.

[24] Starr, J. I., et al. "Measurement of serum proinsulin-like material: cross-reactivity of porcine and human proinsulin in the insulin radioimmunoassay." *The Journal of laboratory and clinical medicine* 91.4 (1978): 683-692.

[25] Matthews, D. R., et al. "Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man." *Diabetologia* 28.7 (1985): 412-419.

[26] Pollex, Rebecca L., and Robert A. Hegele. "Genetic determinants of the metabolic syndrome." *Nature Clinical Practice Cardiovascular Medicine* 3.9 (2006): 482-489.

[27] Boduła, Andrzej, Michał Wdowczyk, and Rajmund Adamiec. "Rola białkowej fosfatazy tyrozynowej 1B (PTP-1B) w rozwoju insulinooporności The role of protein tyrosine phosphatase (PTP-1B) in insulin resistance." *Postepy Hig Med Dosw.(online)* 59 (2005): 203-207.

[28] Dahlman, I., et al. "No association of reported functional protein tyrosine phosphatase 1B 3′ UTR gene polymorphism with features of the metabolic syndrome in a Swedish population." *Journal of Internal Medicine* 255.6 (2004): 694-695.

[29] Pal, Aparna, et al. "PTEN mutations as a cause of constitutive insulin sensitivity and obesity." *New England Journal of Medicine* 367.11 (2012): 1002-1011.

[30] Kenner, Kathleen A., et al. "Protein-tyrosine phosphatase 1B is a negative regulator of insulin-and insulin-like growth factor-I-stimulated signaling." *Journal of Biological Chemistry* 271.33 (1996): 19810-19816.

[31] Florez, Jose C., et al. "Association testing of the protein tyrosine phosphatase 1B gene (*PTPN1*) with type 2 diabetes in 7,883 people." *Diabetes* 54.6 (2005): 1884-1891.

[32] Florez, Jose C., Joel Hirschhorn, and David Altshuler. "The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits." *Annual Review of Genomics and Human Genetics* 4.1 (2003): 257-291.

[33] Mackawy, A. M. H., E. Abd-Alfarag Ahmed, and M. E. H. Badawy. "Association of protein tyrosine phosphatase 1B (*PTPN1*) gene polymorphisms (1023C> A and 467T> C) with type 2 diabetes: A case-control study." *J Clin Med Genomics* 3.135 (2016): 2.

[34] Weng, Jianping, et al. "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* 26.10 (2003): 2957-2957.

[35] Santaniemi, Merja, Olavi Ukkola, and Y. A Kesäniemi. "Tyrosine phosphatase 1B and leptin receptor genes and their interaction in type 2 diabetes." *Journal of Internal Medicine* 256.1 (2004): 48-55.

[36] Wanic, K., et al. "Lack of association between polymorphisms in the gene encoding protein tyrosine phosphatase 1B (*PTPN1*) and risk of Type 2 diabetes." *Diabetic Medicine* 24.6 (2007): 650-655.

[37] Bento, Jennifer L., et al. "Association of protein tyrosine phosphatase 1B gene polymorphisms with type 2 diabetes." *Diabetes* 53.11 (2004): 3007-3012.

[38] Mok, Andrea, et al. "A single nucleotide polymorphism in protein tyrosine phosphatase PTP-1B is associated with protection from diabetes or impaired glucose tolerance in Oji-Cree." *The Journal of Clinical Endocrinology & Metabolism* 87.2 (2002): 724-727.

[39] Di Paola, Rosa, et al. "A variation in 3′ UTR of hPTP1B increases specific gene expression and associates with insulin resistance." *The American Journal of Human Genetics* 70.3 (2002): 806-812.

[40] Palmer, Nicholette D., et al. "Association of protein tyrosine phosphatase 1b gene polymorphisms with measures of glucose homeostasis in Hispanic Americans." *Diabetes* 53.11 (2004): 3013-3019.

[41] Cheyssac, Claire, et al. "Analysis of common *PTPN1* gene variants in type 2 diabetes, obesity and associated phenotypes in the French population." *BMC Medical Genetics* 7.1 (2006): 44.

[42] Echwald, Søren M., et al. "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 (2002): 1-6.

[43] Spencer-Jones, Nicola J., et al. "Protein Tyrosine Phosphatase-1B Gene *PTPN1*." *Diabetes* 54.11 (2005): 3296-3304.

[44] Kipfer-Coudreau, S., et al. "Single nucleotide polymorphisms of protein tyrosine phosphatase 1B gene are associated with obesity in morbidly obese French subjects." *Diabetologia* 47.7 (2004): 1278-1284.

[45] Gouni-Berthold, I., et al. "The Pro387Leu variant of protein tyrosine phosphatase-1B is not associated with diabetes mellitus type 2 in a German population." *Journal of Internal Medicine* 257.3 (2005): 272-280.

[46] Olivier, Michael, et al. "Single nucleotide polymorphisms in protein tyrosine phosphatase 1β (*PTPN1*) are associated with essential hypertension and obesity." *Human Molecular Genetics* 13.17 (2004): 1885-1892.

[47] Rondinone, Cristina M., et al. "Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis." *Diabetes* 51.8 (2002): 2405-2411.

[48] Joy, Tisha, et al. "Genetics of metabolic syndrome." *Current Diabetes Reports* 8.2 (2008): 141-148.