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Original Article

Peroxisome proliferator-activated receptor- γ polymorphism (rs1801282) is associated with obesity in Egyptian patients with coronary artery disease and type 2 diabetes mellitus



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ABSTRACT

Objective: Peroxisome Proliferator-Activated Receptor- γ (PPAR- γ) gene is one of the possible genes linking diabetes mellitus (DM) with coronary artery disease (CAD). The aim of this study is to clarify whether PPAR- γ Pro12Ala polymorphism is associated with the development of CAD in type 2 diabetic patients and to evaluate PPAR- γ Pro12Ala polymorphism genetic distribution in type 2 DM (T2DM) Egyptian subjects.

Methods: PPAR- γ Pro12Ala polymorphism was determined by Real-Time PCR in serum of 405 subjects classified into 4 groups; T2DM patients (n = 105), T2DM with CAD (n = 100), CAD patients (n = 100) and healthy controls (n = 100).

Results: The PPAR- γ Pro12Ala polymorphism was associated significantly with T2DM with CAD (group2) (OR = 3, 95% CI = (1.5–6); p = 0.001). In this study, T2DM with CAD complications carrying the PPAR- γ Pro12Ala polymorphism had higher BMI than those without the PPAR- γ Pro12Ala polymorphism (p < 0.0001). CAD patients carrying PPAR- γ Pro12Ala polymorphism had considerable insulin resistance features. Plasma paraoxanase 1 (PON1) level was considerably reduced among our 3 studied groups in comparison to control group (p < 0.001).

Conclusions: PPAR- γ Pro12Ala polymorphism might represent a novel risk factor for CAD in T2DM.

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1. Introduction

Diabetes mellitus (DM) is a long-standing metabolic disease that became a great health issue all over the world [1]. The wide-spread of type 2 DM (T2DM), which is the main type of diabetes, has increased significantly with the expansion of obesity and the lifestyle of low physical activity [2].

The main cause of T2DM is indistinct, but the disease has a multigenic nature evident by the interaction of environmental and genetic factors [3]. The chronic diabetic vascular problems are counted as the main cause of illness and death in diabetic patients [1]. Patients with DM have a high hazard of early

atherosclerosis [4,5]. T2DM has more than twice increased in the occurrence of cardiovascular diseases-related death [6].

Peroxisome Proliferator-Activated Receptor- γ (PPAR- γ) gene is located on chromosome 3(3p25), PPAR- γ presents in two isoforms, γ 1 and γ 2 represent major isoforms and γ 3 and γ 4 represent minor forms [7]. The PPAR- γ is major element in atherosclerosis because they are combined with insulin resistance features, such as obesity and diabetes [8]. Furthermore, PPAR- γ has a critical role in adipose tissue formation and subcellular metabolism of arterial wall macrophage foam cells [9,10]. The PPAR- γ controls insulin responsiveness by transcriptionally stimulating adipocyte-specific genes implicated in insulin signaling, lipid storage, fatty acid uptake, and glucose uptake [9]. PPAR- γ Pro12Ala polymorphisms (rs1801282) have been associated with metabolic and cardiovascular death [8,11].

The PPAR- γ rs1801282C > G polymorphism, a single nucleotide polymorphism (SNP) in exon 2 of PPAR- γ , encodes a proline →

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alanine substitution at amino acid residue 12 (Pro12Ala). This mutation reduces the transcription of PPAR- γ 2 [12]. The PPAR- γ rs1801282C>G polymorphism has been extensively investigated and was found to be correlated with the risk of cardiovascular diseases and T2DM [13–16]. But some studies have reported contradictory results [17]. Another studies reported the Pro12Ala polymorphism was protective against diabetes in Caucasians but not in South Asians [18,19].

This study was aiming to clarify whether PPAR- γ Pro12Ala polymorphisms (rs1801282) are associated with the development of coronary artery diseases (CAD) in patients with T2DM and to evaluate PPAR- γ Pro12Ala polymorphisms genetic distribution in T2DM Egyptian subjects.

2. Materials and methods

2.1. Subjects

Our study included 405 subjects recruited from Outpatients'Clinic of the National Research Centre, National Egyptian Institute of Diabetes, and Coronary Care Unit (CCU) of Ain Shams Hospital, Cairo Egypt. All participants answered a questionnaire used for collecting socioeconomic data, as well as family history of diabetes and other diseases. Anthropometric measurements were performed: weight (kg), height (cm), body mass index (BMI) (kg/m²), waist circumference (cm). Hypertension was defined as systolic blood pressure (SBP) \geq 140 mmHg, diastolic blood pressure (DBP) \geq 90 mmHg or under antihypertensive drugs and were measured with a mercury column sphygmomanometer [20]. According to American Diabetes Association (ADA) [21]; DM is diagnosed when fasting blood glucose (FBG) level \geq 126 mg/dl or 2 hours post prandial (2HPP) \geq 200 mg/dl or random plasma glucose (RBG) \geq 200 mg/dl. Ethics Committee's approval and participants' consents were obtained. Our subjects were classified into:

2.1.1. Normal healthy control group

Included 100 subjects with FBG < 126 mg/dl or 2HPP < 200 mg/dl or RBG < 200 mg/dl. Exclusion criteria were hyperlipidemia, hypertension, CAD, diabetes mellitus, hepatic and renal diseases, endocrine disease, metabolic disorders, autoimmune diseases and those under any medication.

2.1.2. T2DM patients without CAD

Included 105 patients with T2DM without CAD or history of CAD fulfilled the ADA diagnostic criteria for diabetes; FBG level \geq 126 mg/dl or 2HPP \geq 200 mg/dl or RBG \geq 200 mg/dl or under diabetes medication. Hepatic, renal, autoimmune, endocrinal diseases, metabolic disorders and autoimmune diseases were exclusion criteria for diabetic patients.

2.1.3. T2DM patients with CAD

Included 100 patients with T2DM with FBG level \geq 126 mg/dl or 2HPP \geq 200 mg/dl or RBG \geq 200 mg/dl or under diabetes medication and complicated with CAD as ischemic heart disease, CAD (included electrocardiographic (ECG) changes or definite myocardial infarction). Exclusion criteria included heart diseases other than CAD, renal disease, hepatic disease, endocrinal diseases, metabolic disorders and autoimmune diseases.

2.1.4. CAD patients

Included 100 patients with CAD as ischemic heart disease, CAD (included electrocardiographic (ECG) changes or definite myocardial infarction) but without DM. Exclusion criteria included heart diseases other than CAD, renal disease, hepatic disease, endocrinal diseases, metabolic disorders and autoimmune diseases.

2.2. Methods

2.2.1. Lipid analysis and biochemical markers

Venous blood samples after a 10-hours fasting were collected from each subject in a sterile EDTA and plain vacutainer tubes. 2 EDTA tubes blood samples were collected one of them stored at -20°C till DNA extraction for genotyping and the other used for measuring glycated hemoglobin (HbA1c). Blood on the plain tubes was allowed to clot for 30 minutes, and then centrifuged at 3000g for 10 minutes at 4°C . Sera were stored at -20°C till time of analysis.

Measurement of serum levels of FBG and lipid profile [total cholesterol, high density lipoprotein (HDL) cholesterol (HDL-C, mg/dL), and triglycerides (mg/dL)] was performed on automated clinical chemistry analyzer (OLYMPUS AU400). Low density lipoprotein cholesterol (LDL-C) level was calculated using Friedewald formula [22].

2.2.2. Assay of glycated hemoglobin (HbA1c)

HbA1c was estimated using Ion-exchange chromatography technique by STANBIO kits (STANBIO LABORATORY *1261 North* Boerne, TX USA 78006).

2.2.3. Serum paraoxanase 1 (PON1), highly sensitive C-reactive protein (hsCRP) and insulin level

Serum paraoxanase 1 (PON1), highly sensitive C-reactive protein (hsCRP) and insulin level were assessed by enzyme-linked immunosorbent assay (ELISA) following instructions of the kits purchased from Immunospec Co.

2.2.4. Genotyping of PPAR- γ Pro12Ala polymorphisms (rs1801282)

DNA was extracted using QIAamp DNA Mini Kits-50-Catalog no. 51104 supplied by QIAGEN. DNA integrity was determined by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized through GEL documentation (E-Gel[®] Imager System with UV Light Base, Thermo scientific). DNA concentration was determined by NanoDrop 2000 Spectrophotometer (Thermo scientific). PPAR- γ Pro12Ala polymorphisms (rs1801282) were detected by Real-Time polymerase chain reactions (PCR) using the Quantistudio 12 Flex real-time PCR system (Applied Biosystems, CA 94404, USA). PPAR- γ Pro12Ala polymorphisms (rs1801282): (Applied BiosystemsID: C_1129864_10) allele discrimination was performed using the TaqMan[®] genotyping protocol (Applied Biosystems, Foster City, CA, USA). PCR reactions were set up in 20 μl reaction volume including 20–30 ng DNA and 10 μl TaqMan[®] Universal PCR Master Mix in 96-well PCR plates. The PCR assay was carried out according to manufacturer's instructions including one step of 10 min at 95°C followed by 40 cycles of DNA denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Final products were analyzed by TaqMan Genotyper software.

2.3. Statistical analysis

IBM SPSS version 18.0 software (Statistical Package for Social Science) was used to analyze the clinical data. Mean values \pm standard error of the mean (SEM) were used to express quantitative data. To ensure adequate statistical power, we included the allele frequency in the statistical study. Frequency of distributions were estimated for quantitative variables. ANOVA test was used for normally distributed data in more than 2 groups, and non parametric data were compared using Kruskal-Wallis test. Chi square test (χ^2) was used to detect the significance of differences between proportions. Differences were considered significant with p value < 0.05 and considered highly significant when P < 0.01. Deviations from Hardy-Weinberg equilibrium and genotype and Allelic differences

were tested using χ^2 test. The association between diseases and gene polymorphism were tested using multivariate logistic regression analysis and presented as unadjusted odds ratios (OR) with confidence interval (95% CI).

3. Results

3.1. Characteristics of the study population

This study is a case-control comparative study consisted of 305 patients divided into 3 groups: T2DM (n = 105), T2DM with CAD (n = 100), CAD (n = 100) and control group (n = 100). The 3 studied groups showed no statistical significant differences as regards age (p = 0.974), while all other studied parameters (height, weight, waist, BMI, SBP, DBP, FBG, 2HPP, HbA1c, cholesterol, triglyceride (TG), HDL, LDL, CRP, PON1 and insulin (INS)) were highly statistically different among the three studied groups when compared to controls (p of all parameters except INS = <0.001 and INS p = 0.009) (Table 1).

3.2. PPAR- γ Pro12Ala polymorphisms (rs1801282) genotype and allele distribution in patients versus controls

The genotype distributions of all groups were in Hardy-Weinberg equilibrium. Analysis of genotypes and allele distribution of PPAR- γ Pro12Ala polymorphisms (rs1801282) in T2DM, T2DM with CAD, CAD and control subjects is shown in Table 2. There were no statistical significant difference in the allele and genotype frequencies of PPAR- γ Pro12Ala polymorphisms (rs1801282) among the T2DM, CAD and control groups (p = 0.517, 0.35 respectively) however, the statistical significant difference was observed between the T2DM with CAD and control groups (p = 0.019).

3.3. Association between Ala12 allele of the PPAR- γ Pro12Ala polymorphisms and risk of development of CAD in T2DM patients

Our study showed that the Pro12Ala12/Ala12Ala12 (rs1801282) genotypes were statistically significant in T2DM with CAD as these genotypes were 3-fold increased risk to develop CAD in T2DM (OR = 3.0, 95% CI = (1.5–6); p = 0.001). But these Pro12Ala12/Ala12Ala12 genotypes showed no statistical significance difference

in CAD group (OR = 1.6, CI = (0.8–3.5); p = 0.191). Also, Ala12 risk allele in T2DM group showed no statistical significant results (OR = 1.03, 95% CI = (0.5–2.3); p = 0.936) (Table 3).

3.4. Relation of presence PPAR- γ risky allele and anthropometric and biochemical parameters among studied groups

Table 4 shows no significant difference between PPAR- γ genotypes (CC versus CG, GG) regarding all parameters in T2DM group. While in T2DM with CAD there were a statistical significant difference in weight, BMI, waist, SBP, DBP, Cholesterol and LDL (p = <0.001, <0.001, 0.011, 0.001, 0.002, 0.012, and 0.017 respectively) between PPAR- γ genotypes (CC versus CG/GG), however there were no significant difference between PPAR- γ genotypes (CC versus CG/GG) regarding all other parameters (Table 5). The CAD patients showed a significant difference in weight, waist, BMI, SBP, DBP, HbA1c, HDL and LDL (p = <0.001, <0.001, <0.001, <0.001, <0.001, <0.001, 0.019, 0.02, and 0.021 respectively) between PPAR- γ genotypes (CC versus CG/GG), however, there were no significant difference between PPAR- γ genotypes (CC versus CG/GG) regarding all other parameters (Table 6).

3.5. Association between the Ala12 allele 'g' of the PPAR- γ Pro12Ala polymorphism and obesity

The Ala 12 allele 'G' of the PPAR- γ Pro12Ala polymorphism was associated with increased BMI in T2DM with CAD and CAD groups (p < 0.001) (Table 7), while no association was found among T2DM patients as well as between controls (p = 0.749, 0.072 respectively).

3.6. Logistic regression analyses of PPAR- γ Pro12Ala polymorphism

Multivariate logistic regression analysis was done after adjustment of other established risk factors: age, male gender, BMI, and smoking status, this analysis showed that PPAR- γ gene (rs1801282) polymorphism was not an independent risk factor of CAD in T2DM patients (p = 0.1236) although it carries a 3.0-fold increased risk to develop CAD in T2DM (p = 0.001), Also PPAR- γ gene (rs1801282) polymorphism was not an independent risk factor neither T2DM nor CAD patients (p = 0.7478, 0.2366 respectively).

Table 1
Demographic and biochemical variables of the studied population.

	T2DM (N = 105) mean \pm SEM	T2DM with CAD (N = 100) mean \pm SEM	CAD (N = 100) mean \pm SEM	Controls (N = 100) mean \pm SEM	P value
Age (year)	47.89 \pm 1.14	49.53 \pm 0.87	48.94 \pm 0.88	48 \pm 0.95	0.974
Height (cm)	163.47 \pm 0.91	166.93 \pm 1.01	169.83 \pm 0.75	165.6 \pm 1.07	<0.001**
Weight (Kg)	82.17 \pm 1.13	90.51 \pm 1.20	95.08 \pm 1.11	74.12 \pm 1.33	<0.001**
Waist (cm)	94.22 \pm 1.89	109.52 \pm 1.06	102.48 \pm 1.20	88.34 \pm 1.22	<0.001**
BMI (Kg/m ²)	30.9 \pm 0.46	32.58 \pm 0.41	33.15 \pm 0.48	27.26 \pm 0.59	<0.001**
SBP (mm Hg)	127.95 \pm 1.34	143.9 \pm 1.66	148.45 \pm 1.63	120.6 \pm 1.24	<0.001**
DBP (mm Hg)	84.14 \pm 1.0	89.15 \pm 1.25	92.4 \pm 1.24	80 \pm 1.01	<0.001**
FBG (mg/dl)	175.56 \pm 8.65	207.88 \pm 5.82	93.13 \pm 2.13	91.09 \pm 2.01	<0.001**
2HPP (mg/dl)	207.67 \pm 9.69	245.17 \pm 8.34	100.6 \pm 1.96	97.82 \pm 2.21	<0.001**
HbA1c %	8.04 \pm 0.22	8.34 \pm 0.15	5.58 \pm 0.08	5.15 \pm 0.07	<0.001**
CHOL (mg/dl)	206.73 \pm 4.72	207.9 \pm 5.20	209.55 \pm 6.10	163.78 \pm 3.35	<0.001**
TG (mg/dl)	172.91 \pm 10.19	192.26 \pm 7.49	171.27 \pm 6.38	102.51 \pm 3.41	<0.001**
HDL (mg/dl)	50.69 \pm 1.47	34.5 \pm 0.88	33.18 \pm 0.93	49.16 \pm 0.99	<0.001**
LDL (mg/dl)	122.11 \pm 3.79	133.45 \pm 5.01	141.2 \pm 5.82	92.96 \pm 3.54	<0.001**
CRP (mg/L)	129.02 \pm 7.87	117.29 \pm 5.92	120.38 \pm 4.64	70.14 \pm 3.81	<0.001**
INS (mIU/L)	15.58 \pm 2.37	11.33 \pm 2.44	13.99 \pm 1.65	6.86 \pm 0.89	0.009
PON1 (mIU/mL)	31.05 \pm 1.04	25.82 \pm 1.31	25.87 \pm 0.76	82.94 \pm 3.36	<0.001**

T2DM: Type 2 diabetes mellitus, T2DM with CAD: Type 2 diabetes mellitus with coronary artery diseases, CAD: coronary artery diseases, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, 2HPP: 2 hours post prandial, HbA1c: heamoglobin A1c (glycated heamoglobin), CHOL: cholesterol, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, CRP: c-reactive protein, INS: insulin and PON1: paraoxenase 1.

The mean difference is significant at the 0.05 level (2-tailed).

** The mean difference is highly significant at the 0.01 level (2-tailed).

Table 2
Genotypes and alleles frequency of PPAR- γ Pro12Ala polymorphisms (rs1801282) in patients and control groups.

	rs1801282 genotype			P value	Allele frequency	
	Pro12Pro12 CC	Pro12Ala12 CG	Pro12Ala12 GG		Pro12Allele C	Pro12Allele G
T2DM (N = 105)	92 (87.6%)	13 (12.4%)	0	0.517	197	13
T2DM with CAD (N = 100)	77 (77%)	14 (14%)	9 (9%)	0.019*	168	32
CAD (N = 100)	82 (82%)	17 (17%)	1 (1%)	0.35	181	19
Controls (N = 100)	89 (89%)	10 (10%)	1 (1%)		188	12

* A statistically significant difference was observed between the T2DM with CAD patients and healthy subjects ($P < 0.05$).

Table 3
Association between Ala12 allele of the PPAR- γ Pro12Ala polymorphism and risk of CAD in T2DM patients.

	Pro12 Allele C	Ala12 Allele G	Odd ratio OR	Confidence interval (CI)	χ^2 (p value)
T2DM (N = 105)	197 (93.8%)	13 (6.2%)	1.03	0.5–2.3	0.006 (0.936)
T2DM with CAD (N = 100)	168 (84%)	32 (16%)	3.0	1.5–6.0	10.3 (0.001**)
CAD (N = 100)	181 (90.5%)	19 (9.5%)	1.6	0.8–3.5	1.713 (0.191)

** The mean difference is highly significant at the 0.01 level (2-tailed).

Table 4
Relation of PPAR- γ Pro12Ala gene polymorphisms and characteristics of subjects among T2DM group.

T2DM (N = 105)	rs1801282 genotype		P value
	G (6.2%) Mean \pm SEM	C (93.8%) Mean \pm SEM	
Height (cm)	164.2 \pm 0.93	163.4 \pm 0.91	0.756
Weight (kg)	87.1 \pm 1.53	81.9 \pm 1.1	0.248
Waist (cm)	100.2 \pm 1.61	93.8 \pm 1.9	0.292
BMI (kg/m ²)	32.2 \pm 0.45	30.8 \pm 0.46	0.243
SBP (mm Hg)	133.1 \pm 1.25	127.6 \pm 1.33	0.152
DBP (mm Hg)	86.5 \pm 0.66	84.0 \pm 1.01	0.371
FBG (mg/dL)	184.4 \pm 8.08	175.0 \pm 8.68	0.704
2HPP (mg/dL)	252.2 \pm 1.01	204.7 \pm 9.6	0.087
HbA1c %	8.7 \pm 0.27	8.0 \pm 0.21	0.229
CHOL (mg/dL)	214.5 \pm 4.34	206.2 \pm 4.74	0.543
TG (mg/dL)	169.6 \pm 8.58	173.1 \pm 10.3	0.905
HDL (mg/dL)	50.2 \pm 1.34	50.7 \pm 1.48	0.893
LDL (mg/dL)	130.4 \pm 3.37	121.6 \pm 3.81	0.415
CRP (mg/L)	1.71 \pm 0.11	1.26 \pm 0.75	0.191
INS (mIU/L)	19.9 \pm 2.09	15.3 \pm 2.39	0.494
PON1 (mIU/ml)	29.5 \pm 1.31	31.2 \pm 1.02	0.57

T2DM: Type 2 diabetes mellitus, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, 2HPP: 2 hours post prandial, HbA1c: heamoglobin A1c (glycated heamoglobin), CHOL: cholesterol, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, CRP: c-reactive protein, INS: insulin and PON1: paraoxenase 1.

* The mean difference is significant at the 0.05 level (2-tailed).

** The mean difference is highly significant at the 0.01 level (2-tailed).

For CAD risk in patients with T2DM or even without T2DM; age, BMI, and smoking status were independent risk factors.

Multivariate Logistic regression analysis of PPAR- γ gene (rs1801282) polymorphism with adjustment for age as potential confounding factor demonstrated an independent effect of the PPAR- γ gene (rs1801282) polymorphism on BMI in T2DM with CAD ($p = 0.0257$) and CAD patients ($p = 0.0065$) but not for T2DM.

4. Discussion

The incidence of DM is increasing worldwide. From 1985 till 2014 the worldwide load of DM is increased from 30 million to 382 million, with the current trends this rate will continue to increase [23].

Cardiovascular diseases (CVD) and DM are closely linked. The most common cause of death in diabetic patients is CVD [24]. In United States the rate of death from CVD in patients with DM in adults is 1.7 times more than patients without DM, as there is an increased risk of myocardial infarction (MI) and stroke [25].

Table 5
Relation of PPAR- γ Pro12Ala gene polymorphisms and characteristics of subjects among T2DM with CAD group.

T2DM with CAD (N = 100)	rs1801282 genotype		P value
	G (16%) Mean \pm SEM	C (84%) Mean \pm SEM	
Height (cm)	167.3 \pm 1.06	166.9 \pm 1.0	0.801
Weight (kg)	100.8 \pm 1.2	88.6 \pm 1.09	<0.001*
Waist (cm)	113.2 \pm 0.8	108.8 \pm 1.08	0.011
BMI (kg/m ²)	36.1 \pm 0.41	31.9 \pm 0.37	<0.001*
SBP (mm Hg)	155.8 \pm 2.15	141.6 \pm 1.45	0.001*
DBP (mm Hg)	97.7 \pm 1.66	87.5 \pm 1.08	0.002*
FBG (mg/dL)	202.5 \pm 5.57	208.9 \pm 5.86	0.567
2HPP (mg/dL)	229.4 \pm 7.30	248.2 \pm 8.48	0.243
HbA1c %	8.4 \pm 0.18	8.3 \pm 0.15	0.798
CHOL (mg/dL)	228.9 \pm 4.69	203.9 \pm 5.2	0.012
TG (mg/dL)	203.8 \pm 7.3	190.1 \pm 7.5	0.34
HDL (mg/dL)	35.9 \pm 1.23	34.2 \pm 0.8	0.336
LDL (mg/dL)	152.7 \pm 5.35	129.8 \pm 4.86	0.017
CRP (mg/L)	1.22 \pm 0.055	1.17 \pm 0.059	0.656
INS (mIU/L)	9.0 \pm 2.11	11.8 \pm 2.5	0.552
PON1 (mIU/ml)	26.7 \pm 0.83	25.7 \pm 1.38	0.694

T2DM: Type 2 diabetes mellitus, T2DM with CAD: Type 2 diabetes mellitus with coronary artery diseases, CAD: coronary artery diseases, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, 2HPP: 2 hours post prandial, HbA1c: heamoglobin A1c (glycated heamoglobin), CHOL: cholesterol, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, CRP: c-reactive protein, INS: insulin and PON1: paraoxenase 1.

* The mean difference is highly significant at the 0.01 level (2-tailed).

** The mean difference is significant at the 0.05 level (2-tailed).

Peroxisome proliferation-activated receptor (PPAR) has three isotypes, namely α , β and γ [26,27]. PPAR- γ gene is one of the possible genes linking DM with CVD as it has a significant role in glucose and lipid metabolism and also involved in many metabolic disorders as DM, CAD, and hyperlipidemia [28,29]. DM is the main independent risk factor for CVD as it increases the occurrence of CAD and MI related death by two-folds [30,31].

The relationship between CAD in patients with or without diabetes and the presence of PPAR- γ Pro12Ala polymorphism (rs1801282) was done in this study. The idea of this work was built on determining a genetic marker for prediction or early detection of CAD in diabetic patients, and we found that, PPAR- γ Pro12Ala polymorphism (rs1801282) was more frequent in diabetic patients with CAD complications than those with diabetes only, thus PPAR- γ Pro12Ala polymorphism increased the risk of CAD among diabetic patients. Our findings are in agreement with a huge study includes approximately 11500 individuals, from which 3870 are obese and 7625 are diabetic [32]. This study correlated the

Table 6
Relation of PPAR-γ Pro12Ala gene polymorphisms and characteristics of subjects among CAD group.

CAD (N = 100)	G (9.5%) Mean ± SEM		C (90.5%) Mean ± SEM		P value
Height (cm)	169.9 ± 0.75		169.8 ± 0.75		0.929
Weight (kg)	109.4 ± 1.4		93.5 ± 0.94		<0.001*
Waist (cm)	114.1 ± 1.0		101 ± 1.15		<0.001*
BMI (kg/m ²)	38.0 ± 0.53		32.6 ± 0.44		<0.001*
SBP (mm Hg)	160.3 ± 0.77		147 ± 1.56		<0.001*
DBP (mm Hg)	102 ± 1.31		91.4 ± 1.19		<0.001*
FBG (mg/dL)	98.4 ± 1.79		92.6 ± 2.15		0.259
2 HPP (mg/dL)	105.6 ± 1.7		100 ± 1.97		0.235
HbA1c %	5.9 ± 0.044		5.5 ± 0.078		0.019
CHOL (mg/dL)	239 ± 6.44		206.4 ± 6.0		0.027
TG (mg/dL)	190.1 ± 6.3		169.3 ± 6.4		0.176
HDL (mg/dL)	30.4 ± 0.45		33.4 ± 0.96		0.02
LDL (mg/dL)	170.3 ± 5.8		138.1 ± 5.7		0.021
CRP (mg/L)	1.14 ± 0.048		1.21 ± 0.046		0.556
INS (mIU/L)	19.6 ± 1.72		13.4 ± 1.63		0.117
PON1 (mIU/ml)	25.5 ± 0.59		25.9 ± 0.78		0.839

CAD: coronary artery diseases, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, 2HPP: 2 hours post prandial, HbA1c: heamoglobin A1c (glycated heamoglobin), CHOL: cholesterol, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, CRP: c-reactive protein, INS: insulin and PON1: paraoxenase 1.

**The mean difference is highly significant at the 0.01 level (2-tailed).

* The mean difference is significant at the 0.05 level (2-tailed).

PPAR-γ Pro12Ala polymorphism with obesity and other factors of metabolic syndrome in diabetic patients with CVD. Our study shows a significant association between PPAR-γ Pro12Ala polymorphism and weight, BMI, SBP, DBP, and LDL in patients suffering from CAD with or without T2DM, but there is no significance difference is seen in patients with T2DM only. The association between PPAR-γ Pro12Ala polymorphism and increased lipid levels in blood was found in obese Palestinian people with T2DM, suggesting that the PPAR-γ Pro12Ala polymorphism existence may have cardiovascular risk [33]. Thus PPAR-γ Pro12Ala polymorphism is related to obesity in cardiovascular complicated diabetic patients and correlated with metabolic syndrome in patients with CVD without diabetes but not in T2DM patients suggesting that PPAR-γ Pro12Ala polymorphism does not act on glucose metabolism but acts through alter lipid metabolism.

The relation between PPAR-γ Pro12Ala polymorphism and obesity in recent studies is still conflicting, some researchers linked PPAR-γ Pro12Ala polymorphism with waist circumference in patients having T2DM [8], while others did not find an association between PPAR-γ Pro12Ala polymorphism and obesity [34–36].

Both of CAD and T2DM have hyperlipidemia and insulin resistance, and this is associated with the severity of CAD through its detrimental effects on glucose [37,38]. In this study, diabetic patients with CAD complications carrying the PPAR-γ Pro12Ala polymorphism had higher BMI, cholesterol, LDL, SBP and DBP than those without the PPAR-γ Pro12Ala polymorphism. Although we could not demonstrate a significant association of the PPAR-γ Pro12Ala polymorphism with CAD without T2DM, but CAD patients carrying PPAR-γ Pro12Ala polymorphism had considerable insulin resistance features. This is in agreement with Gonz'alezS'anchez and colleagues that associated the polymorphism with higher risk of obesity [39].

In this study PPAR-γ Pro12Ala polymorphism has no direct impact on the development of diabetes disease as there was no significant difference between T2DM and PPAR-γ Pro12Ala polymorphism.

In our study there was reduced frequency of the Ala12 allele, relative to many Caucasian populations, which is concordant with recently published study done on Egyptians [40] and several studies from other Arab region [33,41,42]. This may be referred to the genetic variants shared between our studied groups and the other Arab region population compared with other ethnic groups [41,42]. Furthermore, reduced frequency of the Ala12 allele potentially might point that many of the Egyptian population is at risk of developing T2DM. This is in consistent with several studies that showed the influence of Pro12Ala polymorphism in reduced risk of T2DM in several populations. Previous research studies on populations in Asia and on Caucasian founded that the development of T2DM does not correlate with PPAR-γ Pro12Ala polymorphism but it is associated with protection against diabetes [18,19]. However, Various studies searched the relation between the PPAR-γ Pro12Ala polymorphism and T2DM, reportedly suggest that the Pro12Ala polymorphism is a risk factor in the development of T2DM [43,44]. In consistent with previous studies, we could not observe an influence of the PPAR-γ Pro12Ala polymorphism in T2DM on several features that are associated with metabolic syn-

Table 7
Association between BMI and allele frequency of the PPAR-γ Pro12Ala polymorphism in studied groups:

	BMI (kg/m ²)	Pro12 Allele C (no./%) C = 197	Ala12 Allele G (no./%) G = 13	P value
T2 DM (N = 105)	Normal: <27	35 (97.2%)	1 (2.8%)	0.749
	Overweight: 27–29	60 (93.8%)	4 (6.3%)	
	Obese: 30–34	68 (91.9%)	6 (8.1%)	
	Extreme obesity: ≥35	34 (94.4%)	2 (5.6%)	
T2DM with CAD (N = 100)		C = 168	G = 32	<0.001**
	Normal: <27	24 (100%)	0 (0%)	
	Overweight: 27–29	27 (96.4%)	1 (3.6%)	
	Obese: 30–34	79 (87.8%)	11 (12.2%)	
CAD (N = 100)		C = 181	G = 19	<0.001**
	Normal: <27	14 (100%)	0 (0%)	
	Overweight: 27–29	46 (95.8%)	2 (4.2%)	
	Obese: 30–34	73 (96.1%)	3 (3.9%)	
Controls (N = 100)		C = 188	G = 12	0.072
	Normal: <27	130 (97%)	4 (3%)	
	Overweight: 27–29	32 (88.9%)	4 (11.1%)	
	Obese: 30–34	9 (90%)	1 (10%)	
	Extreme obesity: ≥35	17 (85%)	3 (15%)	

** The mean difference is highly significant at the 0.01 level (2-tailed).

drome such as the waist, BMI, blood pressure, TG levels, cholesterol-HDL and FBG [45,46].

5. Conclusions and recommendations

We concluded that the PPAR- γ Pro12Ala polymorphism plays a role in the development of CAD in obese T2DM. The linkage between T2DM and PPAR- γ Pro12Ala polymorphism must be further investigated in diabetic patients with vascular complications and in different populations for future treatment and preventive measures and to understand better the mechanism of development of DM and its destructive impacts.

Author contributions

NH made the study design, supervised the project, collected and analyzed data and drafted the manuscript. SK participated in the molecular genetic studies, participated in sample collection, data collection and analysis and shared in writing the manuscript. MH participated in the molecular genetic studies and data collection. EA helped in biochemical investigations, molecular genetic studies, helped to draft and submit the manuscript, AH participated in biochemical investigations and molecular genetic studies, NM collected samples and data of cardiac patients, GH participated in collection of samples and data of diabetic patients. This manuscript was revised approved by all authors.

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