ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Mar 15; 6(3):447-455.
https://doi.org/10.3889/oamjms.2018.156
eISSN: 1857-9655
Basic Science

Is the rs1801282 (G/C) Polymorphism of *PPAR - Gamma* Gene Associated with T2DM in Iraqi People?

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Citation: Al-Naemi AH, Ahmad AJ. Is the rs1801282 (G/C) Polymorphism of PPAR - Gamma Gene Associated with T2DM in Iraqi People? Open Access Maced J Med Sci. 2018 Mar 15; 6(3):447-455. https://doi.org/10.3889/oamjms.2018.156

Keywords: PPAR gamma; T2DM; polymorphism; Pro12Ala; Iraqi

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Received: 09-Mar-2014; **Revised:** 12-May-2014; **Accepted:** 14-May-2014; Online first: 14-Mar-2018

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Pro12Ala (rs1801282) is a common polymorphism of the human *PPAR-γ* gene. Studies have demonstrated conflicting results about its association with T2DM worldwide. There are no reports about such possible association among Iraqi people.

OBJECTIVES: This study aims at finding out whether having the mutant allele (Ala12) might be associated with T2DM among Iraqi people.

METHODS: One hundred and ninety-two Arabic Iraqi adult subjects (97 with T2DM and 95 controls) were genotyped using PCR- RFLP. Clinical, anthropometrical and biochemical variables were compared regarding the Pro12Ala genotypes.

RESULTS: About 5.67% of people with diabetes were carriers of the (Ala12) allele versus 9.47% of controls. Allelic and genotypic frequencies were not statistically different among diabetics and controls [(χ2= 1.99, p= 0.16) and (χ2= 2.17, p= 0.14)]. Age, BMI and smoking- but not Pro12Ala - were independent risk factors for T2DM in our subjects. Pro12Ala was not associated with T2DM (Odd's ratio 0.55, 95% CI 0.23- 1.32, p= 0.14).

CONCLUSIONS: Our study revealed a relatively high frequency of the Ala12 allele among Arabic Iraqis. These frequencies did not significantly differ between diabetics and controls indicating the absence of association of Pro12Ala with T2DM among Iraqis.

Introduction

Diabetes Mellitus (DM) is a heterogeneous group of metabolic disorders that occur as an ultimate result of defective endogenous insulin secretion and/or action [1]. The genetic contribution in the pathogenesis of type 2 diabetes mellitus (T2DM) is remarkable given the inheritance is seen in families, the high prevalence rates of the disease in certain ethnic groups compared to others and the difference in the concordance rates when monozygotic twins are compared to dizygotic ones [2] [3]. The genetic background of T2DM has shown to be a cocktail as described by Freeman and Cox who suggested that understanding the basis of the genetic traits of T2DM can help identify new therapeutic targets, which currently represents one of the most promising strategies for the long-term treatment success [4].

Scientists have used their tools for exploring the genetic background of T2DM including “Genome Scans” and “Association studies”. The association studies, including Genome Wide Association Studies (GWAS), are case-control studies that investigate the relationship of particular disease status with certain alleles, genotype (or haplotype) or most commonly a set of single-nucleotide polymorphisms (SNPs) [4] [5] [6] [7]. The GWAS(s) which were first successfully conducted in 2005 is a major tool for identifying different biological pathways, understanding the pathophysiology of several complex (multifactorial) diseases and developing drug therapies [7]. They typically conduct the first analysis in an exploration cohort and then validate the most significant genetic findings (mainly SNPs) in an independent validation cohort [8].Several association studies have identified some key genes for human T2DM susceptibility of which the peroxisome proliferator-activated receptor gamma (*PPAR-γ*) and the *KCNJ11* are the most promising genes [9] [10]. However, using the candidate gene approach, other several genes have been recently confirmed as susceptibility genes like *CAPN10, HNF4A, TCF7L2, BCL11A, MTNR1B,*

NOTCH2/ADAM S30, DCD, VEGFA, GCK, FTO, HHEX/IDE, JAZFI, KCNQ1, CYP3A4, and others [11].

Peroxisome proliferator-activated receptors are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily [12]. Their activation through their ligands binding leads to augment the expression of many genes especially those involved in lipid metabolism and energy homeostasis [13] [14]. There are three well-identified types of *PPARs; PPAR α, PPARδ* (or *PPARβ*) and *PPARγ*. The DNA- binding domains of the three subtypes are about 80% identical [15].

Human *PPAR γ* gene is located on chromosome 3 (3p25), and it spans a genomic segment of around 150 kb. It consists of 9 exons (A1, A2, B and 1-6) that code for the two major isoforms of PPAR γ mRNA and protein (PPAR γ1 and PPAR γ2) via the use of different promoters and alternative splicing [16].

In humans, PPAR γ2 is mainly expressed in the adipose tissue, and it is a critical transcriptional factor in the regulation of adipocytes differentiation and the expression of many genes that are responsible for lipogenesis and insulin signalling [17]. PPAR γ agonists –whether natural like polyunsaturated fatty acids or synthetic like the antidiabetic drugs thiazolidinediones- have been shown to improve the overall body insulin sensitivity in a variety of insulin resistant experimental animals and diabetic humans [18] [19].

A common C→ G polymorphism of *PPAR-γ* gene results in a proline to alanine substitution at the codon 12 of the gene (Pro12Ala) with a reduced transcriptional activity of the resultant protein [20] [21]. The minor allele (Ala12 allele) frequency of this common gene variant is widely variable worldwide. It was found to range from 5.9% to 21.6% among Caucasian ethnicities, and from 1.7% to 9.3% among people of East Asian descent such as Chinese and Japanese [22].

There has been conflicting results about the association of Pro12Ala mutation of *PPAR- γ* gene with T2DM in different populations [23] [28]. We hypothesize that this variant is linked to the risk of T2DM in Iraqi people as well due to its possible impacts on insulin action and/or signaling pathways and that the inconsistent findings concerning its association with diabetes around the world is a reflection of the multifaceted interactions between this polymorphism and various environmental factors (including the different fatty acids contents in people diet world-wide) and/or between this sequence variation and other genetic factors.

In fact, there have been no studies on the association of Pro12Ala (rs1801282) of *PPAR-γ* gene with T2DM in Iraqi people. So, this study aims to find out whether having its mutant allele (Ala12) is associated to T2DM in a representative sample of adult Iraqi people of Arabic ethnicity.

Materials and Methods

This study has been approved by the "Human Research Ethical Committee" of Ninevah Health Directorate, Ninevah, Iraq in April 2013. Written consents were obtained from all participants after explaining the research objectives, plans and procedures during a brief interview and the work was conducted by the ethical standards of Declaration of Helsinki II.

This age, sex and ethnicity matched case-control study was designed to investigate the association of the Pro12Ala mutation of *PPAR-γ* gene with T2DM among 192 adult Iraqi people over a period of 7 months from May 3rd. Through November 30^{th,} 2013. It involved 97 (57males and 40 females) unrelated adult (34-71 years old) Arabic Iraqi T2DM patients with variable duration and onset of diabetes consulting Al-Wafaa Center of Diabetes Care and Education in Western Mosul, Ninevah, Iraq and 95 (51males and 44 females) apparently healthy unrelated non-diabetic Iraqi adult (30-70 years old) subjects with negative family history of diabetes mellitus.

Diabetes mellitus was defined by physician's diagnosis according to the American Diabetes Association (ADA) criteria; a fasting plasma glucose (FPG) level ≥ 7 mmol/l (126 mg/dl), two hours postprandial glucose (2h PG) level ≥ 11.1 mmol/l (200 mg/dl) or use of diabetes medications [29]. Iraq is a multi-ethnic, multi-religious and multi-nationalities country. This study focused only on Arabic residents of different urban areas of Mosul City, Ninevah Province, Northern Iraq without a history of mixed marriages with other ethnicities till the third generation.

The diabetic patients who formed Group (I) were recruited for history taking, physical examination and blood collection, when ready, after overnight fasting. Resting blood pressure was measured in sitting position, and anthropometric measurements were taken including height and weight with shoes off and light clothes. Body mass index (BMI) was calculated as weight (kg)/ height (m)^2. Patients were excluded from the study when having T1DM, previous history of ketoacidosis, other than Arabic ethnicities, history of liver or kidney diseases (or any malignancy) or being involved in another clinical or interventional study.

The subjects who formed Group (II) were basically the relatives of non- diabetic patients admitted for surgical operations at Al-Joumhori Teaching Hospital in Western Mosul in addition to the

members of the nursing staff of the hospital. They have all fulfilled the inclusion criteria and agreed to participate in this study.

To be considered as "Controls", subjects were required to be over the age of 30 years, having a negative personal and family history of diabetes mellitus with their fasting plasma glucose being in the normal range (3.33-5.56 mmol/l) following overnight fasting. Any history of previous abnormal glucose homeostasis and current FPG > 5.56 mmol/l was enough to exclude the subject from the control group. Other exclusion criteria for being among the controls included the history of liver or kidney diseases, history of current or recent participation in an interventional study or drug abuse. History was taken from control subjects; anthropometric parameters were measured, resting blood pressure was recorded, and blood was aspirated.

Plasma glucose was estimated manually at the laboratory of Biochemistry, Mosul Medical College relying on glucose oxidase-peroxidase method, which is highly specific for D-glucose [30] using a kit supplied by Randox Ltd (England). The genetic work has been conducted in the PCR laboratory of Ibn Al-Atheer Teaching Hospital, Eastern Mosul. Genomic DNA was extracted from EDTA-treated whole blood samples using ReliaPrep™ Blood gDNA MiniPrep System (Promega Corporation, USA) following the manufacturer's protocol. All T2DM patients and controls were genotyped for the Pro12Ala of *PPARG* gene using PCR-based restriction fragment length polymorphism (PCR-RFLP) assay as designed by Hamann *et al.* [31]. A 306 bp DNA sequence embracing the ambiguity site (**c**ca→**g**ca) at codon 12 was first amplified using primers supplied by (GenScript, USA) as forward:

5′-GCCAATTCAAGCCCAGTC-3′

and reverse:

5′-CGTCCCCAATAGCCGTATC-3′

Amplification was done over 35 cycles in a final volume of 25 µl using 12.5 µl of 2XQiagen's PCR Master Mix (containing $MgCl_2$ at final concentration of 3mM and 5 units/µL HotStar Taq *Plus* DNA polymerase), 0.5 µl of each of forward and reverse primers (10 µM), 9µl of RNase- free water and 2.5 µl (average 300 ng) of DNA. The thermal conditions used for the amplification of the rs1801282 in our subjects are shown in Table 1.

Table 1: Thermal conditions to amplify Pro12Ala for subsequent RFLP

CONDITION	TEMPERATURE	TIME
Initial denaturation	95˚C	5 min
Denaturation	95˚C	30 sec
Annealing	60˚C	90 sec
Extension	72˚C	30 sec
Final extension	68˚C	10 min
Final volume= 25 µl, cycles= 35		

Following PCR, amplicons (306 bp) were subjected to digestion by a specific restriction endonuclease where the C/G variation creates a restriction site for *HgaI* enzyme. The recognition site for *HgaI* is the following:

5′- G A C G C (N) 5 ▼..3′

3′- C T G C G (N) 10 ▲..5′

Ten-µl of PCR products were digested with 3 units of HgaI (New England Biolabs, UK) in a total reaction volume of 50 µl containing 5 µl of 10X 1.1 NEBuffer (10 mM Bis Tris Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.0 at 25°C) at 37C° for 60 minutes. The reaction was terminated by enzyme inactivation at 65°C for 20 min. DNA fragments were separated on 2% agarose gel stained with SYBR ® Safe DNA Gel Stain (Promega, USA) following standard gel electrophoresis, visualised on the UV transilluminator and photographed. Control DNA samples (wild-type CC, heterozygous mutant CG and homozygous mutant GG) whose genotypes were already confirmed by sequencing (primer extension) were subjected to the same PCR_RFLP reactions, and their digestion products were used in the electrophoretic runs for the confirmation of the samples genotypes.

Statistical Analysis

Standard statistical methods were used to determine the mean, standard deviation (SD) and range (minimum-maximum). One Sample Kolmogorov- Smirnov Test (two-tailed) was used to investigate the normality of distribution of the study variables. Two-tailed unpaired Student's t-test was used to compare the mean values of continuous variables among subjects in different groups when variables were normally distributed or otherwise replaced by Mann Whitney-U test to compare variables that did not follow the normal distribution pattern.

Categorical variables were compared by Chi-square (χ^2), or by using Fisher's exact test if the number of observations within a cell was less than five. Obedience of Pro12Ala genotypes to the Hardey-Weinberg Equilibrium (HWE) was also assessed using χ 2 test. HWE calculator was obtained from http://www.tufts.edu. Logistic regression analysis was used to study the relation between T2DM as the dependent variable and other influencers (like Pro12Ala gene polymorphism) as the independent variables. Mantel- Hanszel test was used to calculate the Odd's ration for the association of Pro12Ala with T2DM and its 95th CI. Differences between observations were considered non- significant at p > 0.0.5. Analyses were carried out using the Statistical Package for the Social Sciences (SPSS Statistics, 17.0).

Results

Among the diabetic patients, using the One-Sample Kolmogorov-Smirnov test, the age, body weight, BMI, systolic blood pressure (SBP) and FPG were normally distributed ($p > 0.05$). However, diastolic blood pressure (DBP) did not follow the pattern of normal distribution ($p < 0.05$). On the other hand, however, both SBP and DBP were not normally distributed among the controls. The results of One-Sample Kolmogorov-Smirnov Test for the characteristics' distribution of both diabetics and controls are displayed in Table 2.

Table 2: The pattern of distribution of the basic characteristics of subjects in both study groups using One- Sample Kolmogorov- Smirnov test.

		Age (years)	Weight (kg)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	FPG (mmol/l)
Diabetics (Group I) (n = 97)	Mean	53.42	81.56	30.14	130	81	9.78
	SD	7.55	11.48	4.87	22	12	3.59
	Kolmogorov-Smirnov Z-value	1.06	0.73	0.69	1.04	1.65	1.10
	p-value	0.22	0.66	0.73	0.23	0.01*	0.18
Non-diabetics (GroupII) (n = 95)	Mean	51.04	74.48	27.73	124	81	4.41
	SD	8.00	17.04	6.22	16	8	0.59
	Kolmogorov-Smirnov Z-value	1.27	0.81	0.73	2.64	2.60	0.67
	p-value	0.08	0.52	0.67	<0.0001*	<0.0001*	0.76

BMI: body mass index; SBP: systolic blood pressure; DBP; diastolic blood pressure; FPG: fasting plasma glucose; SD: standard deviation. *p < 0.05 using One Sample Kolmogorov-Smirnov Test (two-tailed) indicates that variables are not normally distributed.

The variables that were not normally distributed in either group of subjects were transformed to their Log10-values, and the One-Sample Kolmogorov Smirnov Test was repeated one more time. Log10 transformation did not succeed to normalize DBP and SBP values ($p < 0.0001$).There were no statistically significant differences in the mean age, sex distribution, smoking frequency and DBP between the two groups ($p > 0.05$ for all). Non-diabetic controls demonstrated significantly lower mean values of body weight, BMI, FPG and SBP (Table 3).

Table 3: The basic clinical, anthropometric and biochemical characteristics of the diabetic and control subjects

Parameter		Group I (T2DM patients) (n= 97)	Group II (Control subjects) (n = 95)	p-Value
		mean ± SD or n (%)	mean ± SD or n (%)	
Age (years)		53.42 ± 7.55	51.04 ± 8.00	0.05
Sex	Males	54 (55.67)	51 (53.68)	0.40
	Females	43 (44.33)	44 (46.32)	
Body weight (Kg)		81.56 ± 11.48	74.48 ± 17.04	0.001
BMI group (Kg/m^2)	< 25	14 (14.44)	37 (38.94)	<0.01
	25-30	35 (36.08)	25 (26.32)	
	> 30	48 (49.48)	33 (34.74)	
BMI (Kg/m^2)		30.14 ± 4.87	27.73 ± 6.22	0.003
Hypertension	Yes	60 (61.86)	35 (36.84)	<0.001
	No	37 (38.14)	60 (63.16)	
SBP (mmHg)*		130.2 ± 22.8	124.9 ± 16.3	0.04
DBP (mmHg)*		81.3 ± 12.3	81.1 ± 8.67	0.63
Smoking	Yes	44 (45.36)	32 (33.68)	0.09
	No	53 (54.64)	63 (66.32)	
FPG (mmol/L)		9.78 ± 3.59	4.41 ± 0.59	<0.0001

* Using Mann Whitney-U test. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose. Hypertension was defined by having SBP≥ 140 mmHg and DBP ≥ 90 mmHg or use of antihypertensive drugs.

Genotypes frequencies of Pro12Ala (rs1801282) among diabetic subjects and controls were within HWE [(χ2=0.35, p= 0.554) and (χ 2=1.04, p= 0.308)] respectively. The comparisons of the allelic and genotypic frequencies of Pro12Ala mutation of PPAR-γ2 gene between diabetics and controls did not show any statistically significant differences [(χ^2= 1.99, p= 0.16) and (χ^2= 2.17, p= 0.14)] respectively (Table 4).

Table 4: Comparisons of allelic and genotypic frequencies of "Pro12Ala" polymorphism among diabetics and controls

	Allelic Frequency n (%)		Genotypic Frequency n (%)		
	C (Pro)	G (Ala)	CC (Pro/Pro)	GC (Pro/Ala)	GG (Ala/Ala)
Diabetic Patients (Group I) N = 97	183 (94.33)	11 (5.67)	86 (88.66)	11 (11.34)	0 (0.00)
Non- Diabetics (Group II) N = 95	172 (90.53)	18 (9.47)	77 (81.05)	18 (18.95)	0 (0.00)
p- value*	0.16		0.14		

*Using Chi-square test.

The association of the mutant allele of the Pro12Ala polymorphism with T2DM was assessed using logistic regression analysis where diabetes was assigned as the dependent variable while Pro12Ala, age, sex, BMI and smoking were considered as the independent co-variables. The analysis demonstrated that having the Ala12 allele is not associated with T2DM (B= -0.16, P= 0.74). However, age, BMI and smoking were significantly associated with the development of diabetes in the study subjects (Table 5).

Table 5: Logistic regression analysis for the association of Pro12Ala mutation of *PPAR-γ* gene and other co- variables with T2DM

Factor	B- value	p- value
Pro12Ala gene variant	-0.16	0.74
Age	-0.03	0.04
Sex	0.02	0.63
BMI	-0.04	< 0.01
Smoking	-1.03	0.02

*BMI: body mass index. The fitness of model was assessed by R- square estimation (R^2 was 0.45) and by Hosmer and Lemeshow test (χ^2= 13.11, df=8, p-value= 0.11).

The Odds' ratio and its 95% confidence interval (95 % CI) for the association of Pro12Ala mutation with T2DM were calculated using Mantel-Haenszel test. The Pro12Ala was not associated with the risk of developing T2DM where Odd's ratio was 0.55 (95% CI= 0.23-1.32, p= 0.14).

The presence of the Ala12 allele was not associated with any significant differences in the mean values of body weight, FPG, SBP, DBP and BMI in both diabetics and non- diabetic subjects (p> 0.05 for all). However, the Pro12Ala mutation was more prevalent among male non- people with diabetes compared to females in the same group (77.78% vs. 22.22%, p= 0.02). The genotypes distribution by BMI did not show significant differences between Pro/Pro and Pro/Ala carriers in the presence and absence of T2DM (p= 0.91 and 0.72 respectively)

(Table 6).

Discussion

The CCA to GCA variation at codon 12 of the *PPAR-γ2* specific exon is a common sequence variation with a reduced functional capacity as a transcriptional factor for a large number of genes [21], [32]. The minor allele frequency of the Pro12Ala polymorphism of the PPAR-γ gene has found to vary widely across different populations worldwide with its lowest frequency being in people of East Asian descent and highest figures being in Caucasians [22]. In Iraq, and to best of our knowledge, this is the first study that investigates the distribution and the association of this common functional polymorphism with T2DM among adult Iraqi subjects of Arabic descent. Iraq is a multinational and a multi-ethnic country in North-West Asia. This study has focused on a populations' sample of 192 non- related Native Arabic adult Iraqis living in urban areas of Mosul, the biggest city of Ninevah Province which is the second largest province in Iraq.

Table 6: Comparisons of clinical, anthropometric and biochemical variables of people with diabetes and non- people with diabetes by their genotypes of Pro12Ala mutation

		Diabetics (Group I) (n = 97)			Non- Diabetics (n = 95)		
		Pro/Pro (n = 86)	Pro/Ala (n = 11)	p-value	Pro/Pro (n = 77)	Pro/Ala (n = 18)	p-value
Sex	Female	36 (41.9)	7 (63.64)	0.17	40 (51.9)	4 (22.22)	0.02
	Male	50 (58.1)	4 (36.36)		37 (48.1)	14 (77.78)	
Body weight (Kg)		82.06± 11.71	77.64± 8.97	0.16	75.28± 10.22	74.29± 18.32	0.76
BMI (Kg/m^2)		30.23± 5.05	29.40± 3.12	0.41	27.76± 6.56	27.55± 4.54	0.80
BMI Groups	< 25	12 (85.71)	2 (14.29)	0.91	30 (81.08)	7 (18.92)	0.72
	25- 30	31 (88.57)	4 (11.43)		20 (76.92)	6 (23.08)	
	≥ 30	43 (89.58)	5 (10.42)		27 (84.38)	5 (15.62)	
SBP (mmHg)		129.9± 23.0	132.3± 21.5	0.60	124.9± 16.9	125± 13.9	0.78
DBP (mmHg)		81.4± 12.6	80.9± 9.7	0.97	81.0± 8.9	81.7± 7.9	0.82
FPG (mmol/l)		9.87±3.66	9.11± 3.09	0.40	4.51± 0.60	4.35± 0.53	0.62

*Values are expressed as mean ± SD or n(%). Comparisons of continuous variables were made using unpaired Student's t-test (two-tailed) except for SBP & DBP which were compared using Mann Whitney U test. Sex distribution was assessed by Chi-square test while genotypes distribution by BMI was compared by Fisher's Exact test.

We demonstrated lack of statistically significant differences in the allelic and genotypic frequencies of Pro12Ala mutation between diabetic patients and non- diabetic controls. The Odd's ratio of the association between diabetes and this mutation was not significant (p = 0.14). Moreover, the Pro12Ala polymorphism of the human *PPAR gamma* gene was not a significant independent factor in the aetiology of T2DM in our patients (p = 0.74).

These results point towards lack of association between the mutant *PPAR-γ2* gene variant and the development of T2DM among the studied sample of the Iraqi population. Similar to the findings of the current study, other researchers have also revealed no evidence of an association between Pro12Ala and diabetes among different European and Asian populations [33] [34] [35].

Reports from our region, the Middle East, also showed this lack of association between diabetes and Pro12Ala. Among these is a study of Tunisian subjects by Zouari Boussaida *et al.* [26]. Also, in Qatar, Badii *et al.* have found that the frequency of the mutant allele of Pro12Ala among cases of T2DM did not differ significantly from that of controls and that the association of this polymorphism with diabetes was not significant among Qatari consanguineous population [36].

Several previous studies elsewhere have demonstrated a significant association of the Pro12Ala polymorphism with T2DM. Ghoussaini *et al.* found a significant association between PPAR-γ 2 Pro12Ala variant and T2DM [37]. The Pro12Ala polymorphism of *PPARG* gene contributed to the risk of developing T2DM in 554 Indian Sikhs according to a recent study [38]. More recently, a study in 2012 revealed that genetic variants of *PPAR-γ, ADIPOQ* and *HNF4A* genes were individually and jointly associated with T2DM among Hong Kong Chinese people and that the Pro12 allele is the risk allele for both T2DM and coronary heart disease [39].

Although a few studies only observed a higher Ala12 allele frequency among the diabetic patients compared to healthy controls conferring the possible "risky" contribution of the Ala12 allele, the association of the Pro12 allele of the *PPAR-γ* gene with diabetes mellitus was the basic finding of many other studies worldwide including an extensive meta- analysis involving 3000 individuals which showed a 1.25- fold increase in the diabetic risk upon having the wild-type allele [40] [41] [42] [43] [44] [45] [46] [47] [48].

While the exact mechanisms by which the Pro12Ala variant of *PPAR-γ* gene is associated with diabetes is not fully understood, it seems that the Ala12 allele improves the peripheral insulin sensitivity by reducing the release of insulin-desensitizing free fatty acids, tumor necrosis factor-alpha and resistin in addition to the increased release of adiponectin (an insulin-sensitizing hormone). These effects will ultimately enhance peripheral glucose uptake and inhibit the hepatic glucose production [16].

The inconsistency of the results of different association studies all over the world- including the current work- may be partially explained by the heterogeneity of the participant's ethnicity. It is assumed that around 14% of the between-studies variances may be attributed to differences in the ethnicity [21].

Also, the differences in the allelic and the genotypic frequencies of the Pro12Ala gene variant among different populations, and thus their contribution to the Odd's ratio of this mutation as a predictor for T2DM may also be referred to the possible overestimation or underestimation of the mutations' frequency. Different scientists used

different genotyping techniques to investigate the mutation like PCR-RFLP, TaqMan probes, DHPLC and others which may have different sensitivities. Differences in the study designs and sample sizes may affect the results.

Small sample size in some studies- including this one- may affect the power of the statistical analyses. For this purpose, we conducted a Post- hoc analysis to compute the achieved power of the study using the G*Power software 3.0.10 (Exact- proportions). The power was 0.69 assuming an expected rate of the risky allele (Pro12) to be 0.95 among people with diabetes and 0.85 among controls. Our small sample size, thus, represents a major study limitation that may affect our conclusions regarding the association of the polymorphism with diabetes and its subsequent impact on the diabetic phenotypes in our patients.

Other possible influences that perhaps stand behind these discrepancies may be possible gene-gene interactions and some gene-environmental (including gene-diet) interactions. Controls chosen to participate in many of the association studies including the current one may not have been selected so perfect. So many of these studies relied on self-reported controls with random glucose measurement to rule out diabetes. A better controls selection would probably require conducting oral glucose tolerance test (OGTT).

These divergent results may also be explained by the possible effect of the Pro12Ala gene variation on other factors that contribute to the aetiology of diabetes like insulin secretion and/or action in response to free fatty acids and physical activity [49] [50]. People in our community are consuming a lot of fat in their diet and are currently experiencing a more westernised pattern of life than before with overtly reduced average daily physical activity. These interactions may attenuate the susceptibility to diabetes in the presence of the less risky Ala12 allele. For this reason, we highly recommend emphasising on having detailed information on diet and physical activity measures in parallel with the Pro12Ala mutation analyses in Iraqi people in the future investigations.

PPAR-γ2 gene variant plays important roles in the adipocytes differentiation and energy metabolism. Its variants like Pro12Ala substitution are expected to be associated with obesity [51]. Our subjects carrying the Ala allele, whether diabetics or controls, got lower mean body weight and BMI respectively than those carrying the wild-type allele only although these comparisons did not reach the level of statistical significance.

In our study, the differences in the frequency of the Ala12 allele by the BMI categories were not statistically significant and in comparison to lean subjects, obese subjects did not demonstrate a statistically significant lower frequency of the mutant allele (p = 0.74). Wang *et al.* indicated that the Pro12Ala variant of the *PPAR-γ* gene is associated with obesity in Chinese Han people. Their diabetic and non- diabetic carriers of Ala12 carriers were significantly leaner than non- Ala12 carriers. However, they demonstrated that the frequency of the Ala12 allele was significantly lowest at BMI> 28 kg/m^2 [44].

Again the variance in the sample size (192 vs 3146) can partially explain this inconsistent association of Pro12Ala gene polymorphism with obesity in both studies. Another reason can be the different definitions of obesity adopted by the two studies where our study relied on the BMI cut points by WHO [52] which are basically derived from data of Western population, while Wang *et al.* considered obesity at BMI≥ 28 kg/m^2 and overweight at BMI ≥ 24 kg/m^2. Asians may have more cumulative risks for the development of cardiovascular diseases at these lower BMI values [53] [54].

Despite that the current work demonstrated a lower mean BMI among (Ala) allele carriers with and without diabetes mellitus, there are some reports that non- diabetic carriers of the mutant allele of Pro12Ala gene variant may have a higher tendency to gain weight over time [36], [55]. Lindi *et al.* demonstrated that carrying the Ala12 allele would be rather in favour of increased insulin sensitivity and thus weight gain happens without the development of diabetes. In our study, the lower mean fasting glucose level among the diabetic and the non- diabetic heterozygotes compared to the wild-type homozygotes may reflect this improved insulin sensitivity and supports this hypothesis. However, direct measurement of insulin sensitivity (as by IVOGTT or hyperinsulinaemic euglycemic clamp) or its surrogate measurements (like by HOMA, or QUICKI) would better affirm this.

PPAR-γ is an important player in blood pressure control [10]. In our study, the Pro/Ala heterozygotes demonstrated a higher mean value of SBP compared to the Pro/Pro homozygotes although this relation has not been adjusted for other independent risk factors like age and BMI as this comparison did not reach the level of significance. By our results, other investigators have demonstrated an association between the Ala12 allele and the elevation of systolic blood pressure and/or diastolic blood pressure [56] [57]. A reasonable explanation for this association between hypertension and Pro12Ala polymorphism is the one already suggested by Sugawara *et al.* [58] who showed that PPAR gamma activation could downregulate the expression of the angiotensin II receptor gene in the vascular smooth muscle cells among experimental animals. This effect may be abolished when the PPAR-γ protein loses a part of its transcriptional capacity upon having functional polymorphisms in its coding gene as in case of rs1801282.

Our study revealed a relatively high frequency of the Ala12 allele and Pro/Ala genotype of Pro12Ala

polymorphism among Iraqi people of Arabic descent. However, these frequencies were not significantly different among diabetics and non- diabetic control subjects. The Pro12Ala mutation failed to predict T2DM in our subjects and did not behave as an independent risk factor for the development of diabetes possibly due to the limited study power because of the relatively small sample size. However, the risk of diabetes in our patients was evidently related to other independent variables like age, body mass index and smoking. Although non-significant, Ala12 carriers exhibited lower mean body weight and BMI regardless of diabetes. The lower mean blood glucose level among these subjects may reflect higher peripheral insulin sensitivity than what may Pro/Pro carriers have. Also, the Ala12 allele carriers may be prone to systolic hypertension possibly due to the reduced transcriptional capacity of the mutant *PPAR-γ* gene variant.

Acknowledgements

The authors would like to thank Ninevah Health Directorate and the staff of PCR lab in Ibn Al-Atheer Teaching Hospital for allowing us accessing the lab's equipment and facilities. Our sincere gratitude goes to Dr Teguh H. Sasongko and Fatemeh Hayati, Human Genome Center, USM, Malaysia for their advisory comments. Finally, we are grateful to all subjects who gave their consents to participate in this study.

References

1. Barnett PS and Braunstein GD. Diabetes Mellitus. In: Carpenter CCJ, Griggs RC, Benjamin IJ (Eds). Andreoli and Carpenter's Cecil Essentials of Medicine, 7th. ed., Saunders Elsevier, Philadelphia, USA, 2007: pp 676- 707.

2. Knowler WC, Pettitt DJ, Saad MF, Bennett PH. Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. Diab Metab Rev. 1990; 6: 1-27. https://doi.org/10.1002/dmr.5610060101

3. Florez JC, Hirschhorn, Altshuler D. The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. Ann Rev Ge nomics Hum Genet. 2003; 4: 257- 291. https://doi.org/10.1146/annurev.genom.4.070802.110436 PMid:14527304

4. Freeman H and Cox RD. Type-2 diabetes: a cocktail of genetic discovery. Hum Mol Genet. 2006; 15 (2): R 202- 209.

5. The International HapMap Consortium. The International HapMap Project. Nature. 2003; 426 (6968): 789–796. https://doi.org/10.1038/nature02168 PMid:14685227

6. The International HapMap Consortium. A haplotype map of the human genome. Nature. 2005; 437 (7063): 1299–1320. https://doi.org/10.1038/nature04226 PMid:16255080 PMCid:PMC1880871

7. Fareed M, Afzal M. Single nucleotide polymorphism in genome-wide association of human population: A tool for broad spectrum services. Egyp J Med Hum Genet. 2013; 14: 123- 134. https://doi.org/10.1016/j.ejmhg.2012.08.001

8. Clarke GM, Anderson CA, Pettersson FH, et al. Basic statistical analysis in genetic case- control studies. Nat Protoc. 2011; 6(2): 121- 133. https://doi.org/10.1038/nprot.2010.182 PMid:21293453 PMCid:PMC3154648

9. Schwanstecher C and Schwanstecher M. Nucleotide sensitivity of pancreatic ATP-sensitive potassium channels and type 2 diabetes. Diabetes. 2002; 51(Suppl.3): S358- S362. https://doi.org/10.2337/diabetes.51.2007.S358 PMid:12475775

10. Barroso I, Gurnell M, Crowley VE, et al. Dominant negative mutations in human PPAR γ associated with severe insulin resistance, diabetes mellitus and hypertension. Nature. 1999; 402 (6764): 880- 883. https://doi.org/10.1038/47254 PMid:10622252

11. Simonis-Bik AM, Nijpels G, Van Haeften TW, et al. Gene variants in the novel Type 2 diabetes loci CDC123/CAMK1D, THADA, ADAMTS9, BCL11A, and MTNR1B affect different aspects of pancreatic β-cell function. Diabetes. 2010; 59: 293- 301. https://doi.org/10.2337/db09-1048 PMid:19833888 PMCid:PMC2797936

12. Mangelsdorf DJ, Thummel C, Beato M, et al. The nuclear receptor superfamily: the second decade. Cell. 1995; 83(6): 835-839. https://doi.org/10.1016/0092-8674(95)90199-X

13. Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J Lipid Res. 1996; 37(5): 907-925. PMid:8725145

14. Perissi V and Rosenfeld MG. Controlling nuclear receptors: the circular logic of cofactor cycles. Nat Rev Mol Cell Biol. 2005; 6(7): 542- 554. https://doi.org/10.1038/nrm1680 PMid:15957004

15. Tachibana K, Kobayashi Y, Tanaka T, et al. Gene expression profiling of potential peroxisome proliferator-activated receptor (PPAR) target genes in human hepatoblastoma cell lines inducibly expressing PPAR isoforms. Nuclear Receptor. 2005; 3:1–17. https://doi.org/10.1186/1478-1336-3-3 PMid:16197558 PMCid:PMC1262764

16. He W. PPARgamma2 Polymorphism and Human Health. PPAR Res. 2009; 2009:849538. https://doi.org/10.1155/2009/849538 PMid:19390629 PMCid:PMC2669649

17. Stumvoll M and Häring H. The peroxisome proliferator-activated receptor- γ2 Prol 12 Ala polymorphism. Diabetes. 2002; 51(8): 2341- 2347. https://doi.org/10.2337/diabetes.51.8.2341 PMid:12145143

18. Olefsky JM. Treatment of insulin resistance with peroxisome proliferator- activated receptor γ agonists. J Clin Invest. 2000; 106(4): 467- 472. https://doi.org/10.1172/JCI10843 PMid:10953021 PMCid:PMC380259

19. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nature Medicine. 2004; 10(4): 355- 361. https://doi.org/10.1038/nm1025 PMid:15057233

20. Masugi J, Tamori Y, Mori H, Koike T, Kasuga M. Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator- activated receptor –γ2 on thiazolidinedion- induced adipogenesis. BBRC. 2000; 268(1): 178- 182. PMid:10652233

21. Yen CJ, Beamer BA, Negri C, et al. Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. Biochem Biophys Res Commun. 1997; 241: 270-274. https://doi.org/10.1006/bbrc.1997.7798 PMid:9425261

22. Gouda HN, Sagoo GS, Harding AH, et al. The association between the peroxisome proliferator- activated receptor-γ2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: A HuGE review and meta- analysis. Am J Epidemiol. 2010; 171: 645-655. https://doi.org/10.1093/aje/kwp450 PMid:20179158 PMCid:PMC2834889

23. Srámková D, Kunesová M, Hainer V, et al. Is a Pro12Ala

polymorphism of the PPARgamma 2 gene related to obesity and type 2 diabetes mellitus in the Czech population? Ann N Y Acad Sci. 2002; 967: 265- 273. https://doi.org/10.1111/j.1749-6632.2002.tb04282.x PMid:12079854

24. Muller YL, Bogardus C, Beamer BA, Shuldiner AR, Baier LJ. A functional variant in the peroxisome proliferator-activated receptor γ2 promoter is associated with predictors of obesity and type 2 diabetes in Pima Indians. Diabetes. 2003; 52 (7): 1864-1871. https://doi.org/10.2337/diabetes.52.7.1864 PMid:12829658

25. Doney ASF, Fischer B, Cecil JE, et al. Association of the Pro12Ala and C1431T variants of PPARG and their haplotypes with susceptibility to Type 2 diabetes. Diabetologia. 2004; 47(3): 555-558. https://doi.org/10.1007/s00125-003-1323-1 PMid:14730381

26. Zouari Bouassida K, Chouchane L, Jellouli K, et al. The peroxisome proliferator activated receptor gamma2 (PPARgamma2) Pro12Ala variant: lack of association with type 2 diabetes in obese and non obese Tunisian patients. Diabetes Metab. 2005; 31(2): 119- 123. https://doi.org/10.1016/S1262-3636(07)70177-5

27. Meshkani R, Taghikhani M, Larijani B, et al. Pro12Ala polymorphism of the peroxisome proliferator- activated receptor-gamma 2(PPARgamma-2) gene is associated with greater insulin sensitivity and decreased risk of type 2 diabetes in an Iranian population. Clin Chem Lab Med. 2007; 45(4): 477- 482. https://doi.org/10.1515/CCLM.2007.095 PMid:17439324

28. Thorsby PM, Midthjell K, Gjerlaugsen N, et al. Comparison of genetic risk in three candidate genes (TCF7L2, PPARG, KNCJ11) with traditional risk factors for type 2 diabetes in a population-based study_ the HUNT study. Scand J Clin Lab Invest. 2009; 69(2): 282- 287. https://doi.org/10.1080/00365510802538188 PMid:18972257

29. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2010; 33 (Supplement 1): S62-S69. https://doi.org/10.2337/dc10-S062 PMid:20042775 PMCid:PMC2797383

30. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem. 1969; 9(6):24-25. https://doi.org/10.1177/000456326900600108

31. Hamann A, Munzberg H, Buttron P, et al. Missense variants in the human peroxisome proliferator- activated receptor-g2 gene in lean and obese subjects. Eur J Endocrinol. 1999; 141: 90- 92. https://doi.org/10.1530/eje.0.1410090 PMid:10407229

32. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index, and improved insulin sensitivity. Nat Genet. 1998; 20(3):284–287. https://doi.org/10.1038/3099 PMid:9806549

33. Ringel J, Engeli S, Distler A, Sharma AM. Pro12Ala missense mutation of the peroxisome proliferator activated receptor gamma and diabetes mellitus. Biochem Biophys Res Commun. 1999; 254(2): 450- 453. https://doi.org/10.1006/bbrc.1998.9962 PMid:9918859

34. Mancini FP, Vaccaro O, Sabatino L, et al. Pro12Ala substitution in the peroxisome proliferator- activated receptor- γ2 is not associated with type 2 diabetes. Brief genetic report. Diabetes. 1999; 48: 1466- 1468. https://doi.org/10.2337/diabetes.48.7.1466 PMid:10389855

35. Dnawati CW, Nagata M, Moriyama H, et al. A possible association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma2 gene with obesity in native Javanese in Indonesia. Diabetes Metab Res Rev. 2005; 21(5): 465- 469. https://doi.org/10.1002/dmrr.543 PMid:15739197

36. Badii R, Bener A, Zirie M, et al. Lack of association between the Pro12Ala polymorphism of the PPAR-γ2 gene and type 2 diabetes mellitus in the Qatari consanguineous population. Acta Diabetol. 2008; 45: 15- 21. https://doi.org/10.1007/s00592-007-0013-8 PMid:17805473

37. Ghoussaini M, Meyre D, Lobbens S, et al. Implication of the Pro12Ala polymorphism of the PPAR-gamma 2 gene in type 2 diabetes and obesity in the French Population. BMC Med Genet. 2005; 6: 11. https://doi.org/10.1186/1471-2350-6-11 PMid:15784141 PMCid:PMC1084346

38. Sanghera DK, Demirci FY, Been L, et al. PPARG and ADIPOQ gene polymorphisms increase type 2 diabetes mellitus risk in Asian Indian Sikhs: Pro12Ala still remains as the strongest predictor. Metabolism. 2010; 59: 492–501. https://doi.org/10.1016/j.metabol.2009.07.043 PMid:19846176 PMCid:PMC2843807

39. Ho JS, Germer S, Tam CH, et al. Association of the PPARG Pro12Ala polymorphism with type 2 diabetes and incident coronary heart disease in a Hong Kong Chinese population. Diabetes Res Clin Pract. 2012; 97(3): 483- 491. https://doi.org/10.1016/j.diabres.2012.03.012 PMid:22515931

40. De Pablos-Velasco PL, Martínez-Martín FJ, Rodríguez-Pérez F, et al. Prevalence and determinants of diabetes mellitus and glucose intolerance in a Canarian Caucasian population: Comparison of the 1997 ADA and the 1985 WHO criteria. The Guia study. Diabet Med. 2001; 18: 235- 341. https://doi.org/10.1046/j.1464-5491.2001.00451.x PMid:11318846

41. Mori H, Ikegami H, Kawaguchi Y, et al. The Pro12→ Ala substitution in PPAR γ is associated with resistance to development of diabetes in the general population, possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. Diabetes. 2001; 50: 891- 894. https://doi.org/10.2337/diabetes.50.4.891 PMid:11289058

42. Memisoglu A, Hu FB, Hankinson SE, et al. Prospective study of the association between the proline to alaninee codon 12 polymorphism in the PPAR gamma gene and type 2 diabetes. Diabetes Care. 2003; 26: 2915-2917. https://doi.org/10.2337/diacare.26.10.2915 PMid:14514601

43. Rodriguez- Esparragon FJ, Rodriguez- Perez JC, Macias-Reyes A, Alamo-Santana F. Peroxisome proliferator- activated receptor- gamma2 Pro12Ala and endothelial nitric acid synthase-4a/b gene polymorphisms are associated with essential hypertension. J Hypertens. 2003; 21: 1649- 1655. https://doi.org/10.1097/00004872-200309000-00013 PMid:12923396

44. Wang X, Liu J, Ouyang Y, et al. The association between the Pro12Ala variant of the PPARγ2 and Type 2 diabetes mellitus and obesity in a Chinese population. Plos One. 2013 (e71985); 8(8):1-6.

45. Altschuler D, Hirschhorn JN, Klannenmark M, Lindgren CM, Vohl M. The common PPAR Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet. 2000; 26: 76-80. https://doi.org/10.1038/79216 PMid:10973253

46. Evans D, de Heer J, Hagemann C, et al. Association between the P12A and c1431t polymorphisms in the peroxisome proliferator activated receptor gamma (PPAR gamma) gene and type 2 diabetes. Exp Clin Endocrinol Diabetes. 2001; 109: 151-154. https://doi.org/10.1055/s-2001-14838 PMid:11409297

47. Lindi VI, Uusitupa MI, Lindstrom J, et al. Association of the Pro12Ala polymorphism in the PPAR-gamma2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. Diabetes. 2002; 51: 2581-2586. https://doi.org/10.2337/diabetes.51.8.2581 PMid:12145174

48. Malecki MT, Frey J, Klupa T, et al. The Pro12Ala polymorphism of PPARgamma2 gene and susceptibility to type 2 diabetes mellitus in a Polish population. Diabetes Res Clin Pract. 2003; 62: 105-111. https://doi.org/10.1016/S0168-8227(03)00164-5

49. Stefan N, Fritsche A, Haring HU, Stumvoli M. Effects of experimental elevation of free fatty acids on insulin secretion and insulin sensitivity in healthy carriers of the Pro12Ala polymorphism of the peroxisome proliferator- activated receptor- gamma2 gene. Diabetes. 2001; 50: 1143- 1148. https://doi.org/10.2337/diabetes.50.5.1143 PMid:11334419

50. Franks PW, Luan J, Browne PO, et al. Does peroxisome proliferator- activated receptor gamma genotype (Pro12Ala) modify the association of physical activity and dietary fat with fasting insulin level? Metabolism. 2004; 53: 11- 16. https://doi.org/10.1016/j.metabol.2003.08.005 PMid:14681835

51. Mattevi VS, Zembrzuski VM, Hutz MH. Effects of a PPARG gene variant on obesity characteristics in Brazil. Braz J Med Biol Res. 2007; 40(7): 927- 932. https://doi.org/10.1590/S0100-879X2006005000114 PMid:17653445

52. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation (WHO Technical Report Series 894), 2000.

53. Chang CJ, Wu CH, Chang CS, et al. Low body mass index but high percent body fat in Taiwanese subjects: implications of obesity cutoffs. Int J Obesity. 2003; 27: 253- 259. https://doi.org/10.1038/sj.ijo.802197 PMid:12587007

54. Wildman RP, Gu D, Reynolds K, Duan X, He J. Appropriate body mass index and waist circumstances cutoffs for categorization of overweight and central adiposity among Chinese adults. Am J Clin Nutr. 2004; 80: 1129- 1136. https://doi.org/10.1093/ajcn/80.5.1129 PMid:15531658

55. Lindi V, Sivenius K, Niskanen L, Laakso M, Uusitupa M. Effect of the Pro12Ala polymorphism of the PPAR-gamma2 gene on long-term weight change in Finnish non-diabetic subjects. Diabetologia. 2001; 44: 925. https://doi.org/10.1007/s001250100558 PMid:11508283

56. Douglas JA, Edros MR. The peroxisome proliferator- activated receptor-γ2 Pro12Ala variant. Association with type 2 diabetes and trait differences. Diabetes. 2001; 50: 886- 890. https://doi.org/10.2337/diabetes.50.4.886 PMid:11289057

57. Stefanski A, Majkowska L, Ciechanowicz A, et al. Association between the Pro12Ala variant of the peroxisome proliferator-activated receptor- gamma 2 gene and increased 24-h diastolic blood pressure in obese patients with type II diabetes. J Human Hypertens. 2006; 20: 684- 692. https://doi.org/10.1038/sj.jhh.1002040 PMid:16625233

58. Sugawara A, Takeuchi K, Uruno A, et al. Transcriptional suppression of type 1 angiotensin II receptor gene expression by peroxisome proliferator- activated receptor-γ in vascular smooth muscle cells. Endocrinology. 2001; 142: 3125- 3134. https://doi.org/10.1210/endo.142.7.8272 PMid:11416035