

Study of PPARG2 Gene Polymorphism (Pro12Ala) in Iraqi Patients with Type 2 Diabetes Mellitus.

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Abstract

Background: Type 2 diabetes mellitus is a multifactorial and polygenic disease, which is considered as a major life threatening problem all over the world. There has been a worldwide effort in the identification of susceptibility genes for type 2 diabetes mellitus. At present, adequate data are not available dealing with PPARG2 (rs1801282) gene polymorphisms and its association with type 2 diabetes mellitus cases among Iraqi populations. Thus, we conceived the need for further studies to investigate PPARG2 gene polymorphisms and its susceptibility to type 2 diabetes mellitus. The aim of this research is to study association PPARG2 gene polymorphism (Pro12Ala) with T2DM in Iraqi population.

The study included 105 patients with T2DM referring to clinic and 80 healthy controls randomly selected based on WHO guidelines. BMI, FBS, lipid profile and insulin plasma levels were measured, DNA was extracted from blood and genotyped by PCR-RFLP with specific primers to amplify a fragment for digestion with restriction enzyme (*Hpa II*). Multinomial logistic regression was applied to compare the proportions of genotypes or alleles. Obtained results of PPARG2 gene GG, CG and CC genotype frequencies for T2DM individuals that obtained were 93.34% , 6.66% and 0% respectively, while in control group were 98.75% , 1.25% and 0% respectively . The genetic power of this study was 25.9% and according to HWE , $\chi^2= 41.299$, $Pvalue=0.000$ which is considered to be not significant. As a conclusion, there is no association between PPARG2 gene polymorphism and type 2 diabetes mellitus in Iraqi population.

Keywords : Type 2 diabetes mellitus , PPARG2 , PCR-RFLP , Iraq.

الخلاصة

المقدمة: في العراق هناك اعداد متزايدة من الإصابة بمرض السكري حيث تسبب نسب عالية من الخسائر، اثبتت الدراسات الوراثية ان الطفرة الوراثية في موروثه الـ PPARG2 (Pro12Ala) متعلقة بمرض السكري. كما ان هذه الطفرات قد تؤثر على مؤشر كتلة الجسم، مستوى الدهون و حساسية الانسولين. هدف البحث هو دراسة علاقة التباين الجيني لموروثه الـ PPARG2 (Pro12Ala) مع داء السكري النوع الثاني في المجتمع العراقي .

شملت الدراسة 105 مريض بداء السكري و80 من الاصحاء تم اختيارهم عشوائيا استنادا الى المبادئ التوجيهية لمنظمة الصحة العالمية. تم قياس مؤشر كتلة الجسم، مستوى السكر، مستوى الدهون ومستوى الانسولين لكل شخص. الحامض النووي تم استخلاصه من الدم ثم تنميطه وراثيا باستخدام تقنية (RFLP-PCR) باستخدام مهادت محددة لتضخيم جزء من الموروثه ثم تقطيعها باستخدام الانزيم القاطع (*Hpa II*). تم استخدام الانحدار اللوجستي المتعدد لأجل مقارنة الأنماط الجينية وترددات الأنماط الوراثية. النتائج التي تم الحصول عليها تشير ان الأنماط الجينية متماثلة ومختلفة اللواقح قد فشلت في زيادة خطر الإصابة بالسكري بشكل مؤثر عند مقارنتها بالنوع البري الطبيعي. وفي نفس الاتجاه لم تظهر هذه الأنماط الوراثية تأثير مهم على مؤشر كتلة الجسم، مستوى الدهون وحساسية الانسولين , كأستنتاج التباين الجيني لموروثه الـ PPARG2 (Pro12Ala) ليس عامل خطورة للإصابة بالسكري من النوع الثاني في المجتمع العراقي.

الكلمات المفتاحية: داء السكري النوع الثاني، التباين الجيني لموروثه PPARG2، تقنية PCR-RFLP، العراق.

Introduction

Diabetes mellitus (DM) is a global health epidemic, affecting approximately 171 million people in 2000 and projected to affect more than 360 million in 2030 [WHO, 2013]. Approximately 90% of people with DM have type 2 disease (T2DM) [WHO, 2013]. In contrast to T1DM, which is genetically inherited, T2DM has a complex aetiology that appears to involve numerous environmental risk factors and potentially some genetic risk factors. Predicting T2DM risk is important because the disease can severely affect quality of life. T2DM is associated with a broad array of

cardiovascular diseases, including retinopathy, nephropathy, neuropathy, acute myocardial infarction, stroke and atherosclerosis. It is important to diagnose and manage T2DM as early as possible to ensure therapeutic efficacy and avoid more serious long-term complications. The rising prevalence of T2DM and the importance of early detection and management have led many investigators to search for environmental and genetic risk factors for T2DM and T2DM related complications.

Peroxisome proliferator activated receptors (PPAR) constitute a distinct subfamily of the nuclear receptors that are activated by naturally occurring fatty acids [Balasubramanyam, 2000]. PPAR γ gene is located on chromosome 3p25 and encodes a nuclear transcription factor involved in the expression of hundreds of genes. The PPAR γ gene contains 9 exons, spans more than 100 kb, and because of alternative mRNA splicing results in the production of 2 protein isoforms: PPAR γ 1 and PPAR γ 2 [Fajas *et al.*, 1997]. PPAR γ 2 plays a critical role in glucose homeostasis and serves as the molecular target of a class of insulin-sensitizing drugs called thiazolidinediones (TZDs), which are PPAR γ 2 ligands and are widely used for treatment of type 2 diabetes [Florian *et al.*, 2006]. Within a unique domain of PPAR γ 2 that enhances ligand independent activation [Auwerx, 1999], a prevalent Pro12Ala polymorphism has been identified [Yen *et al.*, 1997] that modulates the transcriptional activity of the gene [Masugi *et al.*, 2000] and is also shown to be involved in the pathogenesis of obesity [Beamer *et al.*, 1998; Deeb *et al.*, 1998; Ek *et al.*, 1999].

A study using a family based design to control for population stratification, reported that Ala-allele of the codon 12 polymorphism was associated with decreased risk of type 2 diabetes [Altshuler *et al.*, 2000]. Then, it became apparent that most negative studies had been underpowered and after combining the data from all published studies in a meta-analysis it became evident that Pro12Ala variant was associated with T2DM [Shania, 2013]. Currently, adequate data is not available dealing with PPAR γ 2 gene polymorphisms and its susceptibility with T2DM cases among Iraqi population; thus we conceived the need for further studies on PPAR γ 2 gene polymorphism and its association with T2DM in Iraqi population.

Objectives

1. To study the association PPAR γ 2 gene (Pro12Ala) polymorphism in Iraqi patients with T2DM.
2. To study the impact of this polymorphism on BMI, lipid profile and insulin sensitivity.

Methods

Study subjects

A case-control study of 185 subjects (105 T2DM and 80 control) was conducted to assess the association of SNP Pro12Ala of PPAR γ 2 gene with T2DM in Iraqi society.

Patients group

The patient society included 105 subjects (43 men and 62 women) with T2DM who attended the diabetes center in Al-Sader Medical City, Najaf, Iraq from January 2014 to March 2014.

Inclusion criteria

1. Those patients who were diagnosed by physicians as having T2DM, the criteria to diagnose diabetes were based on WHO guidelines.
2. A subject was said to have diabetes if his/her fasting glucose level was >126 mg/dl (7.0 mmol/l) in addition of symptoms of diabetes.
3. Age of patients was >40 y.

Exclusion criteria

Any subjects diagnosed with T1DM , who are on insulin therapy, and have diabetic complications , cancer , hepatitis , hormonal disorders or other acute or chronic illnesses were excluded from the study.

Control group

The control group includes 80 healthy subjects (36 men and 44 women). They were randomly selected from the people who attend the hospital for checkup also from relatives and colleagues.

Inclusion criteria

1. Fasting plasma glucose < 100 mg/dl.
2. No past medical history of type 2 diabetes.
3. No family history of diabetes in first-degree relatives
4. Matched to patients with regard to gender, age and geographical distribution.
5. Age at examination > 40 y
6. BMI < 30 kg/m² and more than 16 kg/m².
7. TC < 200 mg/dl 8. TG < 150 mg/dl

All cases answer a detailed questionnaire that includes information about age, sex, family history, drug history, medical history and other relevant information, for all subjects' weight, height and BMI had measured. It should be noted that Najaf was one of the big cities of Iraq, and there was no much difference in genotyping distribution from one city to another, therefore our study society could represent the Iraqi society. Informed consent has been taken from all subjects. Kufa Medical College Ethical Committee has approved the study protocol.

Biomarkers

Phenotypes data included: BMI, FBS, fasting insulin and lipid profile.

Genotyping

Peripheral blood samples of T2DM and control groups were collected in EDTA-anticoagulated tubes, and then DNA was extracted from whole-blood samples using the Reliaprep genomic DNA extraction kit (Promega, U.S.A). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (BioDrop, U.K).

Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) for PPARG2 gene using thermocycler (Biometra, Germany). The sequence of primers used [Ek *et al*, 1999] , was : forward primer 5'-CAA GCC CAG TCC TTT CTG TG-3', and the reverse primer was 5'-AGT GAA GGA ATC GCT TTC CG-3'. Amplification was performed in a total volume of 25 µl contained 12.5 µl GoTaq Green Master Mix, (Promega Corporation, Madison, WI), 1.5 µl of each primer (1 Mm final concentration) (OneAlpha, U.S.A), 3.5 µl nuclease free water, and 6 µl of DNA template. Cycling condition was 94°C for 3 min followed by 40 cycles of 94°C for 30s, 53°C for 30s, 72°C for 1 minute , and a final extension of 72°C for 4 min. Amplification product of PPARG2 gene was 236 bp. The product was digested with 12U of restriction enzyme (*HpaII*) (Bioneer) and run on 3% agarose gel. To determine genotyping error rate, we performed random duplication in 20% of the samples.

Statistical analysis

Phenotypes data expressed as mean ±SD and genotypes data expressed as frequencies, student T test used to compare phenotypes data between control and T2DM groups and across genotypes using SPSS windows software (SPSS Inc., Chicago, IL). Genotype and allele frequencies in T2DM and control group were tested by multinomial logistic regression analysis using SPSS.

Results

Clinical and biochemical characteristics of the study subjects were presented in table (1), it exhibited significant differences in BMI, FBS, lipid profile and insulin sensitivity between T2DM and control group.

Table 1: Clinical and biochemical characteristics of study subjects.

Parameters	T2DM subjects	Control subjects	P value
No (M/F)	105 (41/65)	80 (36/44)	0.580
Age (y)	55.85±9	53.52±9.6	0.141
BMI (kg/m ²)	29.43±4.4	24.16±3.2	0.000
FBS (mg/dl)	216.15±68.28	91.45±7.9	0.000
Cholesterol (mg/dl)	256.05±64.46	160.76±29.45	0.000
Triglycerides (mg/dl)	248.17±76.7	113.6±26.08	0.000
VLDL (mg/dl)	49.63±15.43	22.75±5.52	0.000
LDL (mg/dl)	155.63±62.65	65.343±29.94	0.000
HDL (mg/dl)	51.67±14.77	75±13.151	0.000
Fasting plasma insulin (μU/ml)	27.72±19.27	12.05±8.62	0.000
HOMA-IR	15.362±13.1	2.712±1.949	0.000

Genotyping

PCR product of this gene polymorphism (Pro12Ala) was digested by *Hpa II* restriction enzyme, products of digestion were electrophoresed on 3% agarose (75 V and 120-150 min) and then stained with ethidium bromide, later visualized under UV light. Results discovered one band (236 bp) for GG wild type, two bands of three (236, 216 bp) because the 3rd band is too small about 28 bp and could not be captured by (3-4%) agarose gel, these indicate CG heterozygous genotype and there were no bands that indicate CC homozygous genotype in subjects, as shown in figure (1).

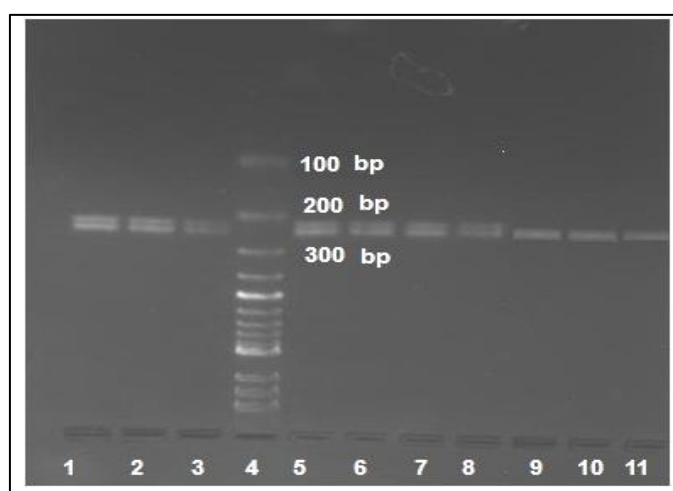


Figure (1) : Results of PPARG2 gene polymorphism (Pro12Ala) product on 3% agarose electrophoresis.

Line 4: DNA marker (100 bp – 3 kb).

Lines 1,2,3,5,6,7 and 8 : CG genotype 236,216 bp.

Lines 9,10 and 11 : GG genotype 236 bp.

Genotype and allele frequencies of PPARG2 gene were shown in table (2), the results demonstrate that PPARG2 gene polymorphism (Pro12Ala) failed to significantly increase the risk of T2DM. The frequency of C allele was higher in T2DM subjects, may be overestimated due to small sample size.

Table (2): Results of genotype and allele frequency of PPARG2 gene polymorphism (Pro12Ala) in patient and control groups.

Pro12Ala C/G	Control N: 80	T2DM N: 105	OR (95% CI)	P value
GG reference	79	98		
CG	1	7	2.364 (0.464-12.035)	0.3
CC	0	0	-	-
Frequency of C allele	1%	3.8%	2.073 (0.541-7.941)	0.288

Clinical characteristics of study subjects according to PPARG2 gene Pro12Ala genotype were shown in table (3). It exhibited failure of Pro12Ala gene polymorphism to affect any of the measured clinical characteristics.

Table (3): Clinical characteristics of T2DM subjects according to PPARG2 gene polymorphism (Pro12Ala).

Clinical Characteristics	CC (n=177)	CG+GG (n=8)	P Value
BMI (kg/m ²)	27.24±4.74	25.078±4.19	0.58
Cholesterol (mg/dl)	212.88±69.83	258.472±74.41	0.77
Triglycerides (mg/dl)	187.3±88.06	249.112±115.7	0.35
LDL(mg/dl)	114.67±67.57	159.588±66.07	0.609
HDL(mg/dl)	62.333±18.15	49.06±15±63	0.72
VLDL (mg/dl)	37.475±17.6	49.82±23.144	0.347
Fasting plasma insulin µU/ml	20.75±17.654	25.311±19.31	0.49
HOMA-IR	9.7±11.680	14.096±13.44	0.35

Discussion

The association between substitution of G (alanine) for C (proline) at codon 12 of PPARG2 and the risk for type 2 diabetes mellitus has been widely studied, Yen *et al.* (1997) firstly reported this polymorphism. Obtained genetic power in the current study was 25.9% and the genotype frequencies of PPARG gene (Pro12Ala) were deviated from Hardy–Weinberg equilibrium (HWE) in both control and T2DM individuals (P= 0.019). These findings matched previous studies such as Bouassida *et al.* and Dehwah *et al.* This deviation from HWE may be attributed to small sample size used in this study.

The current study results showed no homozygous genotype was obtained in this study despite using two restriction enzymes from different companies. Heterozygous CG genotype revealed no significant association to T2DM when it compared to reference wild genotype GG and these findings were reported by previous studies such as French [Maya, 2008], Tunisian [Bouassida, 2005], Chinese [Dehwah, 2008], Qatari [Badii, 2008], Italian [Fabio, 2012] and German [Koch, 1999] populations. Other published studies showed that there is a significant association to increase the predisposition to T2DM like Iranian [Azadeh, 2013], Indian [Syed, 2012], Finns [Andrulionytė, 2004], Russian [Dimitry A, 2010], Americans [Chan, 2013], Egyptians [Salwa, 2009], Taiwanese [Wu S, 2004] and Turkish [Erdogan, 2007] populations. At the same time, other studies showed an association between PPAR γ gene polymorphism and reduction of risk of T2DM, which has been found in different ethnic populations such as Japanese [Horiki, 2004], Korean [Moon, 2005], Scotts [Doney, 2004], Danish [Frederiksen, 2002], Spanish [Soriguer, 2006], and American Caucasians [Radha, 2006].

Minor C allele frequency also failed to show any significant effect on the risk of T2DM when compared to control group which is consistent to previous studies above. Regarding the effect of PPAR γ polymorphism (Pro12Ala) on clinical characteristics, the present study failed to exhibit any significant impact of this polymorphism on BMI, lipid profile, fasting plasma insulin and HOMA-IR (all calculated P values were > 0.05). These results were consistent to previous studies such as German [Koch, 1999], French [Maya, 2008] studies, while inconsistent the results that reported by Egyptians [Salwa, 2009], Iranian [Azadeh, 2013], and Indian [Syed, 2012] studies.

The conflicts among the results of the different studies may be partly explained by the presence of a gene–gene or gene–environment/life style interaction. However, studies of this type of interaction are scant [Federico, 2006]. These discrepancies may be due to problems inherited to the nature of different studies including the sample size which may be regarded as a limitation in the present study.

Conclusion

PPAR γ 2 gene polymorphism (Pro12Ala) is not associated with increased risk of T2DM in Iraqi population and may be another SNP of that gene could be a risk factor.

Recommendations

Including a large sample size to get a sufficient genetic power and to improve the role of PPAR γ 2 in Iraqi population.

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