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COMMON VARIANTS IN THE ADIPONECTIN RECEPTOR 2 (ADIPOR2) GENE IS ASSOCIATED IN T2DM PATIENTS IN WITH AND WITHOUT CARDIOVASCULAR DISEASE AND ADIPONECTIN LEVELS

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ABSTRACT

Background

Patients of type 2 diabetes mellitus (T2DM) are at high risk of developing cardiovascular disease. Two receptor forms, ADIPOR1 and ADIPOR2 , mediate biologic effects of adiponectin. ADIPOR2 is a cell-surface receptor abundantly expressed in skeletal muscle and liver.

Methods

A case-control study conducted to find the association between SNP rs11061971 in T2DM with and without CVD in Al-Najaf Governorate, Iraq. The study included 203 T2DM patients with CVD randomly selected based on World Health Organization (WHO) guideline and 133 T2DM patients without CVD as controls group. DNA was extracted from blood and genotyped by PCR-RFLP by using (BtsCI) enzyme. Multinomial logistic regression was applied to compare the proportions of genotypes and alleles. The odds ratio for risk of developing CVD in T2DM was calculated with and without adjustment for age, sex, and BMI.

Results

Adiponectin receptor R_2 gene polymorphism rs11061971 (homozygous TT and heterozygous AT genotype) was significantly associated with CVD in T2DM patients and the frequency of T allele was higher in T2DM with CVD patients compared to that without CVD.

Conclusion

The SNP of adiponectin receptor 2 gene is involved the pathogenesis of T2DM with CVD in Al-Najaf Governorate, Iraq, Carriers of the homozygous genotype (TT) and heterozygous (AT) genotype of rs11061971 have strong association and increased risk of development of T2DMwith CVD. The T allele frequency of rs11061971 was association with increased risk of development of T2DM with CVD.). R2 adiponectin receptors also play an important role in the metabolism of VLDL cholesterol and TG.

KEYWORDS: Diabetes Mellitus (T2DM), World Health Organization (WHO), Multinomial Logistic Regression, VLDL Cholesterol and TG

INTRODUCTION

Background

Cardiovascular diseases are bigger causes of morbidity and mortality in patients with T2DM. Smoking, high blood pressure, high serum cholesterol and obesity are major risk factors for heart disease in diabetic patients (Bugger *et al*. 2014).

Two receptor forms, ADIPOR1 and ADIPOR2 , mediate biologic effects of adiponectin. In humans, ADIPOR1 is ubiquitously expressed, with highest levels being in the skeletal muscle. ADIPOR2 is a cell-surface receptor abundantly expressed in skeletal muscle and liver (Weigert et al 2008).

The ADIPOR2 gene is located on chromosome 12p13.33, consisting of eight exons. Single nucleotide polymorphisms (SNPs) of the ADIPOR2 have been associated with either insulin resistance or hepatic fat accumulation in various populations (Kilm et al 2009). One of these common genetic variants is rs11061971 which is located in the intron 2 of ADIPOR2.

The marker rs11061971 showed association with both glucose intolerance and T2D in Old Order Amish (Damcott et al 2005). In addition to other studies on Russian population (Potapov et al 2008). Thus, this study is important because it's the first one that investigated this SNP in Iraq Arabic type 2 diabetic patients with CVD.

METHODS

Study Design

A case–control study of 336 persons included two groups (203 type 2 diabetic patients T2DM with cardiovascular disease CVD and 133 type 2 diabetic patients T2DM without cardiovascular disease CVD) randomly selected was conducted to assess the association of SNP (rs11061971) of ADIPOR2 gene.

The study was performed on 336 type 2 diabetic patients (184 male and 152 female).The patients included two groups { 203 diabetic patients with CVD (117 male and 86 female), the patients ages ranged between 45-56 years with mean ± SD (49.03 ± 6.41) } and {133 diabetic patients without CVD (67 male and 66 female), the patients ages ranged between 41- 46 years with mean ± SD (47 ± 6.11) } were included as a control group}. The patient population who attended diabetes center at Al-Sader Medical City, Najaf, Iraq from September 2014 to January 2015. All patients were diagnose by specialist physicians as having type 2 diabetes, were based on WHO guidelines.

Phenotype Measurements

We collected clinical data, such as weight, height, and other data. The BMI was calculated as weight (in kg) divided by the square of height (in m). Serum cholesterol, serum triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were measured. Total serum adiponectin was estimated using an enzyme-linked immune sorbent assay (ELISA).

Genetic Analysis

Blood samples of T2DM with and without CVD were collected in EDTA-anticoagulant tubes, and then DNA was extracted from whole-blood samples using DNA extraction kit (Favorgen, Tawan). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U.K.).

Genotyping was performed by polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP) for adiponectin receptor R_2 gene using thermocycler (Biometra, Germany). The primer sequences were obtained f from (Potapov et al 2008): forward 5'GCATCTGTTCTATCTTCCCATCATAAA3' and reverse 5'GACCCCCACTTTTTTTTCCCCATA3'. Amplification was performed in a total volume of 25 µl which contained 12.5 µl of GOTaq Green Master Mix, (Promega Corpration, Madison, WI), 1.5 µl of each primer (1 Mm final concentration) (One Alpha, U.S.A.), 3.5 µl nuclease free water, and 6 µl of DNA template. Cycling conditions were 96 C° for 3 min followed by 30 cycles of 94 C° for 30s, 52 C° for 30s, 72 C° for 30s, and a final extension of 72 C° for 5 min. Amplification product of adiponectin receptor R_2 gene was 117 bp. The product was digested with 10 U of restriction enzyme (BtsCI) (Promega) and ran on 2% agarose gels.

Statistical Analysis

Student T tests and ANOVA test were used to compare phenotypic data between with and without CVD in T2DM patients using SPSS windows software (SPSS Inc., Chicago, IL). Genotype frequencies were tested for Hardy-Weinberg equilibrium by $\chi 2$ test using online software web-Assotest (www.ekstoem.com). Genetic power was calculated using the online software OSSE (OSSE.bii.a-star.edu.sg). Genotype and allele frequencies in with and without CVD in T2DM patients were tested by multinomial logistic regression analysis with and without adjustment for age, sex and BMI using SPSS.

RESULTS

Anthropometric and biochemical characteristics of study individuals are presented in table 1.

Results of digestion with restriction enzyme (BtsCI) for adiponectin receptor R_2 gene rs11061971 included 117 bp band for wild type (AA) genotype, for the heterozygous genotype (AT) three bands 80, 37 and 117 bp and for homozygous genotype (TT) two bands 80 and 37 bp as shown in Fig. 1. Genotype and allele frequencies of adiponectin receptor R_2 gene are shown in table 2.

Genotype frequencies of rs11061971 were not consistent with Hardy-Weinberg equilibrium in T2DM with CVD individuals (p=0.002) and consistent with T2DM witout CVD individuals (p=0.212). The power of this study to detect a significant difference at level of 0.05 was 81.9%.

The results shown that adiponectin receptor R_2 gene polymorphism rs11061971 (homozygous TT and heterozygous AT genotype) was significantly associated with CVD in T2DM patients and the frequency of T allele was higher in T2DM with CVD patients.

Biochemical characteristics of T2DM with and without CVD individuals according to adiponectin receptor R_2 gene polymorphism (rs11061971) genotypes are shown in tables 3 and 4. It demonstrated that a significant impact on HDL and adiponectin levels was detected for rs11061971.

Table 1: Anthropometric and Biochemical Characteristics of Study Individuals

	T2DM With CVD Subjects N=203	T2DM Without CVD Subjects N=113	P- Value
No (M/F)	203(117/86)	133(67/66)	
Age (y	49.03±6.41	47.33±6.11	0.016
BMI (Kg/m^2)	30.79±5.31	28.94±5.02	0.002
Cholesterol (mg/dl)	232.99±35.29	233.73±34.97	0.851
Triglycerides (mg/dl)	229.89±39.69	226.96±40.58	0.515
VLDL(mg/dl)	45.97±7.93	45.39±8.11	0.515
LDL (mg/dl)	139.58±35.90	139.50±37.20	0.984
HDL (mg/dl)	47.42±6.54	48.83 ±6.78	0.062
Adiponectin (ng/ml)	6.39 ±2.41	7.50 ±1.55	0.000

*Phenotypic data expressed as mean± standard deviation.

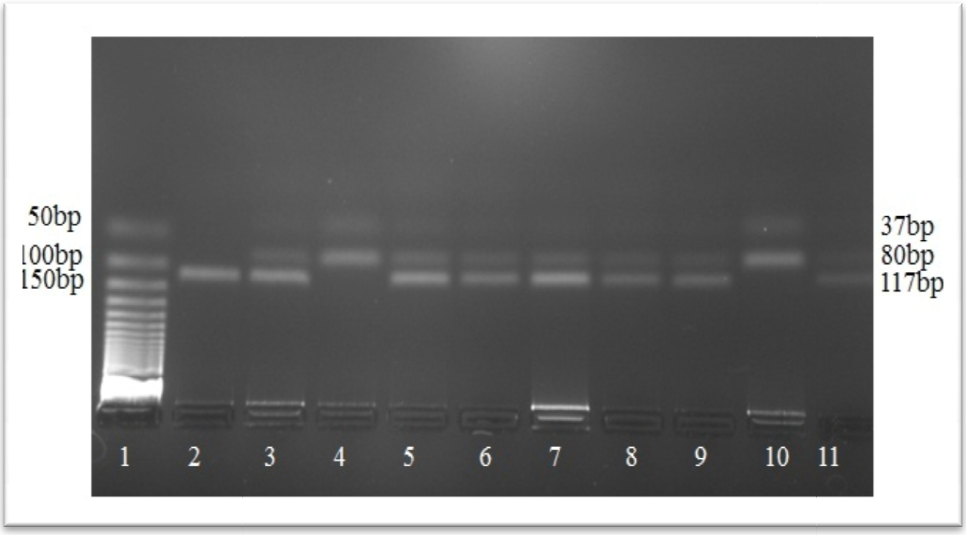

Figure 1: Genotyping Result for Adiponectin Receptor 2 Gene (rs11061971). Marker Line 1. (Lines 2, and 11): for Individuals have the Wild Type (AA) of 117 bp Fragment. (Lines 3, 5, 6, 7, 8, and 9): for Individuals have the Heterozygote (AT) Genotype that Showed Three Fragments with Sizes of 117 bp, 80 bp, and 37 bp.(Lines 4, 10): for Individual have the Homozygote (TT) Genotype Exhibited T wo Fragments of 80 bp and 37 bp Sizes

Table 2: Genotype and Allele Frequency of Rs11061971 Polymorphism of Adiponectin R2 Gene and Association of this Variant In T2DM With and Without CVD in the Study Individuals

T2DM with CVD n=203	T2DM without CVD n=133	Unadjusted OR(95%CI) P-value	Adjusted OR(95%CI) P-value	
Genotype				
Rs11061971				
(A/T)	93	75	Reference	
AA	73	46	1.280(0.793-2.065) 0.049	1.302(0.803- 2.110) 0.284
AT	37	12	2.487(1.212-5.102)	2.490(1.207-5.136) 0.014
TT	147 (36.20%)	70 (26.3%)	0.013	
Frequency of T allele			2.362(1.493-3.73) 0.0002	

Table 3: Biochemical Characteristics Of T2DM With CVD Individuals According to Adiponectin Receptor 2 Gene Polymorphism (Rs11061971) Genotype

Biochemical Characteristics	AA (n=93) Mean ± SD	AT(n=73) Mean ± SD	TT(n=37) Mean ± SD	P-Value
BMI (Kg/m^2)	30.37±5.20	31.16±5.33	31.10±5.49	0.96
Cholesterol (mg/dl)	233.75±33.84	234.67±38.02	227.76±32.68	0.59
Triglycerides (mg/dl)	228.83 ±37.53	229.83 ±41.01	232.65±42.13	0.88
VLDL (mg/dl)	45.76±7.50	45.96±8.20	46.53±8.42	0.88
LDL (mg/dl)	141.11±34.58	141.13±36.19	132.71±36.37	0.43
HDL (mg/dl)	46.87±6.29	47.57±6.19	48.52±7.59	0.41
Adiponectin (ng/ml)	6.44±2.39	6.37±2.33	6.28±2.56	0.94

Table 4: Biochemical Characteristics of T2DM without CVD Individuals According to Adiponectin Receptor 2 Gene Polymorphism (Rs11061971) Genotype

Biochemical Characteristics	AA (n=75) Mean ± SD	AT(n=46) Mean ± SD	TT(n=12) Mean ± SD	P-Value
BMI (Kg/m^2)	28.56±4.87	29.21±5.17	30.30±5.03	0.48
Cholesterol (mg/dl)	232.91±33.19	237.15±37.58	225.73±26.59	0.56
Triglycerides (mg/dl)	226.11 ±39.99	225.82 ±40.15	236.63±44.43	0.68
VLDL (mg/dl)	45.22±7.99	45.16±8.03	47.32±8.88	0.68
LDL (mg/dl)	139.12±34.87	142.84±39.68	129.08±39.35	0.51
HDL (mg/dl)	48.56±6.84	49.14±5.99	49.31±8.87	0.87
Adiponectin (ng/ml)	6.70±2.60	6.08±2.28	7.29±2.09	0.22

DISCUSSIONS

In this study we examined the sequence variation in ADIPOR2 gene contributed to susceptibility to T2DM patients with and without CVD in a Najaf, Iraq population. The markers of ADIPOR2, rs11061971 showed association in T2DM patients with CVD. Similarly, Damcott et al. reported that the allele T of rs11061971 was significantly associated with higher risk of T2DM patients with CVD OR=2.24,95% (1.125-4.490).

Genotype frequencies of adiponectin receptor R_2 gene rs11061971 were not consistent with Hardy-Weinberg equilibrium (HWE) in T2DM with CVD and consistent in without CVD individuals (p=0.002). These findings were also reported by Potapov et al. (2008).

The results of adiponectin receptor R_2 gene polymorphism rs11061971 demonstrated that homozygous genotype (TT) carries have two –fold increased risk of developing CVD when compared with those of the wild type (AA) after adjustment for age, sex, and BMI in addition to the risk in heterozygous (AT) genotype carriers was one folds. Such observations strongly suggested a role of adiponectin receptor R_2 gene polymorphism rs11061971 in the pathogenesis of CVD in patients T2DM in Al-Najaf Governorate, Iraq.

Some ADIPOR2 variants showed association with insulin resistance-related phenotypes such as decreased fasting triglyceride levels, and higher respiratory quotient, i.e. lower rate of fat oxidation. In this study, we found that the ADIPOR2 gene , rs11061971 was significantly associated with higher risk of T2DM individuals with and without CVD. However, our study in consistent with the observations of Potapov et al (Potapov *et al* 2008).

Previously, Dumcott et al. found that the allele T of rs11061971 was significantly associated with higher risk of T2D in Russian population, and genetic variants in the ADIPOR2 gene were found to be associated with type 2 diabetes in the Old Order Amish (Dumcott *et al* 2005). Richardson et al also observed strong associations between ADIPOR2 polymorphisms and plasma triglyceride concentrations may have important implications for atherogenesis and/or dyslipidaemia, owing to the potential influence of ADIPOR2 genetic variation on triglyceride rich lipoprotein metabolism in Mexican Americans (Richardson *et al* 2006). Furthermore Collins et al suggests ADIPOR2 variants are unlikely to be major risk factors for type 2 diabetes and insulin resistance in UK Europid populations, although more detailed analyses of gene variants may be required to exclude a potential minor role of these genes in insulin resistance and glucose homeostasis (Collins et al 2007).

Daimon et al who indicated hypoadiponectinemia to be a risk factor for the development of type 2 diabetes mellitus in Japanese population (Daimon et al., 2003). Baratta et al. and Vendrell et al. also found decreased levels of serum adiponectin concentrations in obese and diabetic subjects and significant inverse associations with some are some possible explanations for the association between T2DM and measures of insulin resistance (Baratta et al. 2004, Vendrell et al.2004). There serum adiponectin concentrations. The lower levels of adiponectin seen in diabetic patients are believed to be associated with the disorder of metabolism of glucose and lipid in diabetes (Lu et al.2006, Karbowska and Kochan, 2006). Diabetic obese patients demonstrate more deteriorated glucose metabolism exhibited by impaired glucose tolerance, or impaired fasting glucose and had also higher serum free fatty acids, higher total and LDL-cholesterol, higher triglycerides, and lower HDL levels, which could also be contributing factors to the lower adiponectin levels.

Previous reports showed that plasma adiponectin levels are affected by multiple factors including gender, age and lifestyle (Kadowaki etal.2006). In this study we elucidate the impact of gender on adiponectin levels in T2DM and control subjects. Female subjects have significantly higher adiponectin levels compared to male subjects in both T2DM patients with CVD and without CVD was consider as control groups. Our observations of the influence of gender suggest that adiponectin production is also related to factors independent of body weight. At any particular body size or body weight, adiponectin concentrations are greater in women than in men. Further, in men and women pair-matched for age, BMI, adiponectin concentrations were greater in women (Zoccali et al. 2002).

Findings from various studies indicate a positive correlation between circulating adiponectin levels and HDL cholesterol concentrations. It is possible that adiponectin regulates HDL cholesterol concentration, independent of BMI and insulin resistance. (Barrett et al 2009). There is an inverse relationship between hormone levels and TG levels. TG is able to reduce adiponectin plasma concentrations (Qiao et al 2008). R2 adiponectin receptors also play an important role in the metabolism of VLDL cholesterol and TG. Our findings suggest that there is an inverse relationship between VLDL cholesterol, LDL cholesterol, TC, LDL concentrations and serum adiponectin levels (Maruyama et al 2009).

Other factors rather than adiponectin SNPs have been shown to regulate adiponectin levels. A diet rich in whole grain and fat was shown to produce increased adiponectin levels (Mantzoros et al 2006, Mantzoros et al., 2005). Physical activity was also shown to influence adiponectin, with high levels of physical activity shown to elevate adiponectin levels (Yu et al., 2009). Nelson et al. was reported in which adiponectin levels are altered independently of ADIPOQ polymorphisms after dietary supplementation with a-linolenic acid (Nelson et al., 2007).

CONCLUSIONS

The SNP of adiponectin receptor 2 gene is involved the pathogenesis of T2DM with CVD in Al-Najaf Governorate, Iraq, Carriers of the homozygous genotype (TT) and heterozygous (AT) genotype of rs11061971 have strong association and increased risk of development of T2DMwith CVD. The T allele frequency of rs11061971 was association with increased risk of development of T2DM with CVD. . R2 adiponectin receptors also play an important role in the metabolism of VLDL cholesterol and TG.

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